

The Relation between Hydrogen Ion
Concentration, Inorganic Salt Con-
centration, and the Rate of
Locomotion in *Amoeba*
proteus

A dissertation submitted to the Board of University Studies of the
Johns Hopkins University in conformity with the requirements
for the degree of Doctor of Philosophy

BY
ROBERT F. PITTS

JUNE, 1932

Reprinted from the JOURNAL OF CELLULAR AND COMPARATIVE PHYSIOLOGY
Vol. 3, No. 4, October, 1933; Vol. 4, No. 2, February, 1934;
Vol. 4, No. 4, June, 1934



THE RELATION BETWEEN INORGANIC SALT CON- CENTRATION, HYDROGEN ION CONCENTRATION AND PHYSIOLOGICAL PROCESSES IN AMOEBA PROTEUS

I. RATE OF LOCOMOTION, GEL/SOL RATIO, AND HYDROGEN ION CONCENTRATION IN BALANCED SALT SOLUTIONS

R. F. PITTS AND S. O. MAST

Zoölogical Laboratory, Johns Hopkins University

ONE FIGURE

(Received for publication June 28, 1933).

INTRODUCTION

There have been numerous investigations concerning respectively the effects of hydrogen ions and salts on various physiological processes, but there have been relatively few concerning the effect of either of these in relation to that of the other.

Lillie ('06) found in observations on the epithelial cells of the gills of *Mytilus edulis* that a solution of sodium chloride, isotonic with sea water, is less injurious in high than in low hydrogen ion concentrations. Loeb ('11) maintains that the injurious effect of acids on eggs of *Fundulus* is decreased if the sodium chloride concentration is increased. Osterhout ('12) comes to a similar conclusion in reference to *Laminaria*; as does also Armstrong ('28) in reference to eggs of *Fundulus*. Barth ('29) asserts that in carbon dioxide free sea water the hydrogen ion concentration necessary for coagulation of the cytoplasm in the eggs of *Arbacia* is higher for inorganic than it is for organic acids, but that in solutions of sodium chloride isotonic with sea water, it is practically the same.

All these investigations clearly show that the action of hydrogen ions is correlated with the salts present, but they give little if any information concerning the interrelation between this action and the kind and the concentration of the salts present.

Farr and Chalkley obtained results which directly concern this interrelation. Farr ('28) observed that as the hydrogen ion concentration decreases, the rate of growth of root hairs of Georgia collards in solutions of CaCl_2 increases to a maximum, then decreases to a median minimum, after which it again increases to a maximum and then decreases to zero; but that the concentrations of hydrogen ions in which the maximum and the minimum rates of growth occur depend upon the concentration of CaCl_2 . Chalkley ('30) found that as the hydrogen ion concentration decreases, the lethal temperature for *Paramecium* increases to a maximum, then decreases to a median minimum, after which it increases to a second maximum, but that the hydrogen ion concentrations for the maximum and the minimum vary greatly with the kinds of salt present.

The results obtained by Farr consequently indicate that the effect of hydrogen ions on physiological processes is specifically correlated with the concentration of the salts present and those obtained by Chalkley that it is specifically correlated with the kind of salts present.

Pantin ('23) and Hopkins ('26) studied the effects of variation in hydrogen ion concentration on locomotion in *Amoeba*. Pantin working on marine limax amoebae used sea water, Hopkins working on *Amoeba proteus* used a modified Ringer solution. Mast and Prosser ('32) investigated the relation between rate of locomotion and gelation in *Amoeba proteus* and hydrogen ion concentration in a given salt solution, and the relation between these processes and salt concentration in a given hydrogen ion concentration. However, the interrelation between these processes and hydrogen ion concentration and salt concentration in different salt solutions was not ascertained in any of these investigations. It is the purpose

of this series of papers to present detailed information concerning these and other correlations, the present paper being specifically concerned with the relations as they exist in a balanced salt solution.

MATERIALS AND METHODS

Organisms. *Amoeba proteus* (Leidy) was used exclusively in the experiments considered in the following pages. The amoebae were cultured in Chalkley solution¹ containing rice. By occasionally removing a small amount of old culture fluid and replenishing it with fresh, the hydrogen ion concentration was kept fairly constant around pH 6.6.

Apparatus. By means of a bath (Pitts, '32) a temperature of $23 \pm 0.5^\circ$ was continuously maintained in all the experiments made. The small dishes inserted in the bath were made from 50 cc. Pyrex glass beakers by cutting off the bottoms.² The edges were ground flat so that a small glass cover could be sealed on. This was found necessary to prevent changes in hydrogen ion concentration during the course of the experiment whenever solutions more alkaline than pH 6.2 were used.

Measurements of hydrogen ion concentrations were for the most part made with the use of a quin-hydrone electrode. Whenever a colorimetric method was used, either Sørensen's phosphate or McIlvaine's phosphate-citrate buffers were used as standards.

Preparation of solutions. All chemicals used were Kahlbaum purest. Salts were carefully dried and weighed; the calcium phosphate, hydroxide and chloride were standardized by the method of Kramer and Tisdall; magnesium chloride was standardized as chloride by the method of Van Slyke; and

¹ The composition of Chalkley solution follows: 80 mgm. NaCl, 4 mgm. NaHCO₃, 4 mgm. KCl, 4 mgm. CaCl₂, 2 mgm. CaH₄(PO₄)₂, 2 mgm. Mg₃(PO₄)₂·4H₂O, and water to 1 liter.

² Hopkins ('29) maintains that the rate of locomotion in *Amoeba* varies with the nature of the substratum, and that it is more nearly constant on either quartz or Pyrex glass than on common glass. Pyrex glass was, therefore, used in the following observations.

the sodium and potassium hydroxides were standardized against constant boiling point hydrochloric acid.

In order to vary the hydrogen ion concentration and at the same time keep the metallic cation concentration constant, a primary phosphate-hydroxide buffer system was used in which the concentration of the cation was identical in the phosphate and in the hydroxide. Hence on mixing the two, no change in metallic cation concentration occurred, no matter what the proportions were. By varying the proportions of the phosphate and hydroxide, a graded series of hydrogen ion concentrations was obtained extending from pH 5.0 to 8.0. The concentration of the cation was varied by changing its concentration in both the phosphate and hydroxide, always, however, keeping it from same in both.

Immediately after preparing a given dilution of the phosphate and hydroxide, a 50-cc. sample of the phosphate was pipetted into a flask and an electrometric titration made, titrating to E.M.F. values corresponding to the hydrogen ion concentrations used. Then to prepare a solution of a given hydrogen ion concentration all that was necessary, was to refer to the titration curve, ascertain the amount of base necessary and to add this amount to 50 cc. of the acid phosphate. As soon as each solution was prepared its hydrogen ion concentration was checked electrometrically and adjusted if necessary. After the completion of the experiment, the solution was removed from the test dish and its hydrogen ion concentration again checked electrometrically or colorimetrically. No evident change occurred in any of the experiments except those in which alkaline solutions were used. Solutions of pH 8.0 or 7.8 shifted in the acid direction during a period of 3 hours to an extent of from 0.05 to 0.3 pH units depending on the concentration of the buffer. If the dishes were not vaseline sealed, the shift was much greater. Results obtained in experiments in which any considerable shift occurred were rejected.

Observation. About 500 amoebae were removed from vigorous cultures and put into watch glasses. They were then

allowed to settle and attach, after which the culture fluid was removed with a pipette. About 5 cc. of redistilled water³ was now poured on and removed with a pipette.⁴ This was repeated until the animals had been in four changes of redistilled water. During this time most of the amoebae assumed a radiate form. These were removed and distributed in four Pyrex glass dishes each containing about 5 cc. of the solution under consideration. This solution was changed three times, then the dishes were sealed with vaseline, after which the amoebae (about 100 in each dish) were left for an hour for adjustment and to become monopodal.

The rate of locomotion was ascertained as follows: A dish containing amoebae in the desired experimental solution was inserted in the temperature bath and left 10 minutes for adjustment of temperature. The bath was then placed on the stage of the microscope and a strictly monopodal specimen⁵ selected. The image of this specimen was now, with a camera lucida, projected on black paper, and the position of the posterior end marked with a white pencil at minute intervals during a period of from 5 to 7 successive minutes.⁶ Another monopodal specimen was then selected and the process repeated. After measurements were made on about five monopodal specimens, the dish was removed from the bath and another substituted, after which the process was repeated.

The total length of a line in millimeters joining the mid-points of these sketches of the posterior end divided by the number of minutes of measurement gives the rate of locomotion of the projected image in millimeters per minute. To

³ Triple distilled water from a tandem Pyrex glass still (Mast, '29) was used in washing the amoebae and in the preparation of all solutions.

⁴ This type of treatment involved a minimum of handling of the amoebae with a pipette and therefore minimum mechanical injury.

⁵ A strictly monopodal amoeba is defined as an elongated cylindrical animal, smooth in outline, which moves continually by the projection of a single pseudopod. Pitts ('33) demonstrated that animals of this form move at a fairly constant velocity with a minimum of variation between different measurements on the same animal and between different animals in the same medium.

⁶ Measurements of rate of locomotion were made under a 40 mm. objective and 15X oculars.

obtain the rate of locomotion of the amoebae in micra per minute this value is multiplied by 13.2.

After the rate of locomotion of five amoebae had been measured as described above the gel/sol ratio was ascertained as described by Mast and Prosser ('32, p. 335).

It would appear logical first to establish the relation between hydrogen ion concentration, rate of locomotion and gel/sol ratio in a solution as nearly like the culture medium as possible. Consequently, since the amoebae used in this investigation were cultured in Chalkley solution, the various hydro-

TABLE 1

Composition of the solution used to ascertain the effect of hydrogen ion concentration on rate of locomotion and gel/sol ratio in a balanced salt solution. To obtain different hydrogen ion concentrations the acid and the alkaline components were mixed in different proportions.

Note that the concentration of Na, K, Ca and Mg remains constant no matter what the proportion of the two components is.

In the second series of experiments the concentration of all the salts was increased five times

ACID COMPONENT		ALKALINE COMPONENT		MOLAE RATIO
NaH_2PO_4	0.00150N	NaOH	0.00150N	Na 60
KH_2PO_4	0.00010N	KOH	0.00010N	K 4
$\text{CaH}_4(\text{PO}_4)_2$	0.00010N	$\text{Ca}(\text{OH})_2$	0.00010N	Ca 2
MgCl_2	0.00005N	MgCl_2	0.00005N	Mg 1

gen ion concentrations tested were obtained by modifying this solution. Two solutions, an acid and an alkaline solution, were prepared as described above. They were mixed in various proportions so as to produce a series of solutions which differed in hydrogen ion concentration but were the same in cation concentration and ratio (table 1). These solutions will hereafter be designated balanced salt solutions. The results obtained in observations on rate of locomotion and gel/sol ratio in amoebae in them are presented in table 2 and figure 1.

RATE OF LOCOMOTION

Table 2 shows that the variation in rate of locomotion was remarkably low resulting in relatively small probable errors.

It shows that as the hydrogen ion concentration increased the average rate of locomotion increased rapidly from 148.9 μ per minute at pH 8.0 to a maximum at pH 7.5, then decreased slightly to a median minimum at pH 7.0, after which it increased to a maximum at pH 6.2, then decreased, at first gradually, and then very rapidly to zero between pH 5.0 and pH 4.6. It also shows there was remarkably little difference

TABLE 2

The relation between hydrogen ion concentration and rate of locomotion in Amoeba proteus in a balanced salt solution. The mean rate was calculated from the rate of the projected image observed in each individual during 5 to 7 consecutive minutes. To obtain the rate of locomotion in micra per minute, multiply the rate of the projected image by 13.2

pH	NUMBER OF INDIVIDUALS	RATE OF LOCOMOTION				
		Projected image of Amoeba, millimeters per minute				Amoeba
		Mean rate	Per cent probable error	Standard deviation	Coefficient of variation	Mean rate in micra/minutes
4.6		0				0
5.0	55	19.47 $\pm$ 0.26	1.32	2.82 $\pm$ 0.18	14.5 $\pm$ 1.0	257.0 $\pm$ 3.6
5.3	55	19.75 $\pm$ 0.25	1.26	2.74 $\pm$ 0.18	13.9 $\pm$ 0.9	260.7 $\pm$ 3.3
5.6	55	20.47 $\pm$ 0.24	1.15	2.59 $\pm$ 0.17	12.7 $\pm$ 0.8	270.2 $\pm$ 3.2
5.9	56	21.04 $\pm$ 0.18	0.86	2.03 $\pm$ 0.13	9.6 $\pm$ 0.6	277.7 $\pm$ 2.4
6.2	55	21.35 $\pm$ 0.20	0.92	2.16 $\pm$ 0.14	10.1 $\pm$ 0.7	281.8 $\pm$ 2.6
6.5	57	20.62 $\pm$ 0.23	1.11	2.55 $\pm$ 0.15	12.4 $\pm$ 0.8	272.2 $\pm$ 3.0
6.8	55	19.84 $\pm$ 0.21	1.09	2.33 $\pm$ 0.16	11.7 $\pm$ 0.8	261.9 $\pm$ 2.8
7.0	55	19.44 $\pm$ 0.23	1.18	2.52 $\pm$ 0.16	13.0 $\pm$ 0.9	256.6 $\pm$ 3.0
7.2	56	20.06 $\pm$ 0.21	1.06	2.36 $\pm$ 0.15	11.8 $\pm$ 0.8	264.8 $\pm$ 2.8
7.5	55	20.52 $\pm$ 0.22	1.06	2.40 $\pm$ 0.15	11.7 $\pm$ 0.8	270.9 $\pm$ 2.9
7.8	59	16.87 $\pm$ 0.26	1.52	2.92 $\pm$ 0.18	17.3 $\pm$ 1.1	222.7 $\pm$ 3.4
8.0	50	11.28 $\pm$ 0.31	2.75	3.25 $\pm$ 0.22	28.8 $\pm$ 2.1	148.9 $\pm$ 4.1
8+		0				0

in the rate in the hydrogen ion concentrations between pH 5.0 and pH 7.5, the mean rate over this range being 267 μ per minute, with a maximum deviation from this mean of only 11.2 μ or 4.19 per cent.

The rapid decrease in rate at the acid end of the range was largely if not entirely due to the failure of the amoebae to attach in solutions which were more acid than pH 5.0, in spite of the fact that they appeared perfectly normal and ex-

hibited marked streaming of the plasmasol. Owing to disintegration of the amoebae there was no locomotion in solutions more alkaline than pH 8.0.

By examining the table it will be seen that the difference in the mean rates in adjoining hydrogen ion concentrations in the series used was so small that the probable errors, al-

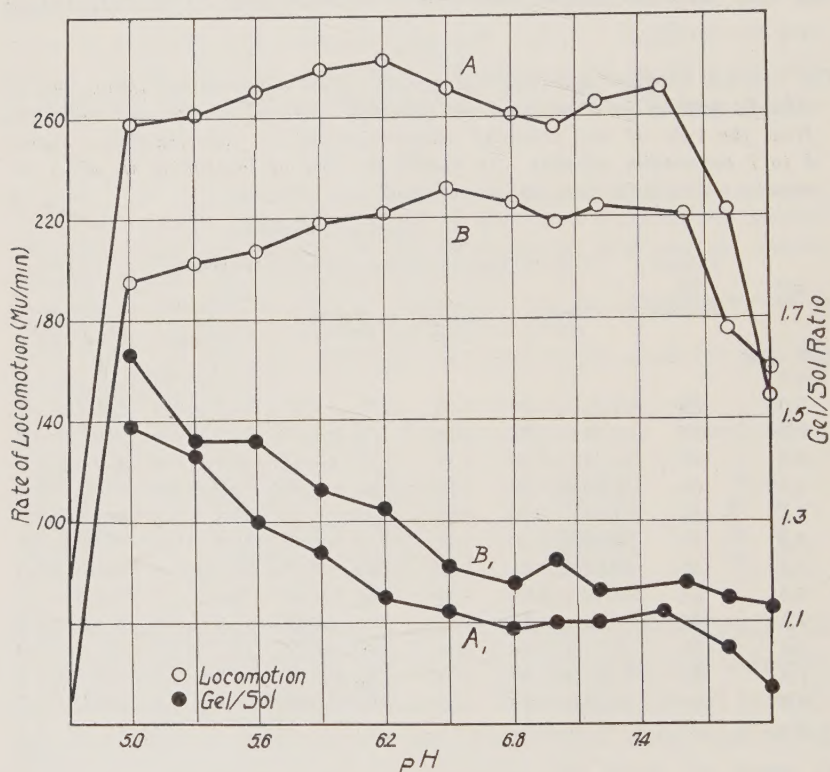


Fig. 1 The relation between rate of locomotion, gel/sol ratio, and hydrogen ion concentration in a balanced salt solution. A, A₁, rate of locomotion and gel/sol ratio in solutions containing salts in concentrations given in table 1; B, B₁, rate of locomotion and gel/sol ratio in salts in concentrations five times as great as those given in table 1. Curve A, mean rates of movement of images of amoebae given in table 2; B, means of the rates of movement of seventeen to twenty-four individuals for from 5 to 7 minutes each in each hydrogen ion concentration; A₁ and B₁, mean gel/sol ratios calculated from results obtained in measurements made on seventeen to twenty-four individuals in each hydrogen ion concentration as described above.

though also relatively very small, show that there was much overlapping of the rates observed in adjoining hydrogen ion concentrations. However, the mean rates of locomotion varied so consistently with changes in hydrogen ion concentration throughout the whole range that the correlation between these means and the hydrogen ion concentration indicated in the table is doubtless valid. The most interesting phase of this correlation, it should be emphasized, is the marked median minimum at neutrality with a maximum rate in the acid range and in the alkaline range.

Table 2 shows that as the hydrogen ion concentration decreased from pH 5.0 the coefficient of variation decreased to a minimum at pH 5.9, then increased to a maximum at pH 7.0, after which it decreased to a second minimum at pH 7.5, and then rapidly increased again. That is, it shows that variability in the rate of locomotion varies inversely with the rate of locomotion throughout the entire range of hydrogen ion concentrations tested.

To ascertain the effect of salt concentration on the relation between rate of locomotion and hydrogen ion concentration, the observations described above were repeated with solutions identical in composition but five times as concentrated. The results obtained are graphically presented in figure 1, in which is also a graphic presentation of the results obtained in the preceding experiments. By referring to this figure it will be seen that the two curves, A and B, representing respectively the relation between rate of locomotion and hydrogen ion concentration in the two salt concentrations used are similar; i.e., that there was a median minimum rate at pH 7.0 in both and a maximum in the acid range and in the alkaline range in both. It will be seen, however, that the difference between the maximum and the median minimum rates was less in the more concentrated solution and that the two maxima were nearer together, indicating decrease in the difference between maximum and median minimum rates with increase in salt concentration. If this is true the median minimum should disappear entirely with increase in salt concen-

tration. This contention was unfortunately not investigated, but the problem concerning the factors involved in the median minimum will be considered in one of the following papers in this series.

The curves referred to above show clearly that the rate of locomotion was markedly higher in the less concentrated solutions, throughout the entire range of hydrogen ion concentrations except at the two extreme limits. This indicates that the rate of locomotion in *Amoeba proteus* is specifically correlated with the salt concentration, but it unfortunately gives no information concerning the optimum concentration. This conclusion is in accord with the contention of Mast and Prosser ('32) concerning the relation between rate of locomotion in *Amoeba proteus* and salt concentration.

The results presented above concerning the relation between rate of locomotion and hydrogen ion concentration do not agree with those obtained by Pantin ('23) in observations on a marine limax amoeba, for he found that as the hydrogen ion concentration decreases, the rate of locomotion increases from zero at pH 5.9 to a maximum at pH 8.5 and then rapidly decreases to zero. They also differ greatly from those obtained by Hopkins ('26) in observations on *Amoeba proteus*, but they are in close agreement with those obtained by Mast and Prosser. These authors say ('32, p. 349): "the rate of locomotion increased to a maximum at about pH 7.4, then decreased to a minimum at about 7, after which it increased to a secondary maximum at about 6.8 and then decreased consistently to the end of the range tested." Thus they find an acid maximum at pH 6.8 which agrees fairly well with the one at pH 6.2 in figure 1 above; and an alkaline maximum at pH 7.4 which agrees very well with the one at pH 7.5. Moreover agreement is complete in so far as the median minimum at pH 7.0 is concerned and the rapid decrease in rate in solutions more alkaline than pH 7.5.

THE GEL/SOL RATIO

The results obtained in observations on the relative thickness of the plasmagel and the plasmasol are presented in figure 1, curves A_1 and B_1 . These curves show that the gel/sol ratio was consistently throughout the entire range of hydrogen ion concentrations tested, considerably higher in the more concentrated of the two series of solutions than in the less concentrated. They show in both of the series of solutions that the gel/sol ratio decreased as the hydrogen ion concentration decreased and that the decrease in this ratio was marked, but that the rate of decrease was somewhat greater in the acid range than in the alkaline range.

These results are in fair agreement with those obtained by Mast and Prosser ('32). They indicate that the more acid the solution and the higher its salt concentration, the more plasmagel in relation to plasmasol, that is, that increase in acidity and in salt concentration induces gelation of plasmasol.

Chambers ('21), Lewis ('23), Leo Loeb ('24), Heilbrunn ('28), Barth ('29), and others, maintain that the viscosity of cytoplasm varies directly with acidity. This contention is in a sense in harmony with the conclusion presented above. It should be stated, however, that the gel/sol ratio is not a direct measure of viscosity; for, though it is a measure of the relative amounts of plasmagel and plasmasol, it gives no indication of the absolute viscosity of either.

By comparing the curves A and B with the curves A_1 and B_1 it at once becomes evident that there is no direct correlation between rate of locomotion and gel/sol ratio in *Amoeba proteus*. This shows that the rate of locomotion in this form is not directly dependent upon the relative thickness of the plasmagel and the plasmasol.

In the preceding discussion it has been assumed that the differences in the rate of locomotion and in the gel/sol ratio considered were due to differences in hydrogen ion concentration of the solutions in which the measurements were made. This assumption is, however, only partially justified, for while the metallic cation concentration was constant throughout the

range, both the ratio of primary to secondary phosphate and the total concentration of phosphate decreased as the hydrogen ion concentration decreased.

The results may, therefore, have been due, at least in part, to change in concentration and in ratio of anions, and to change in osmotic pressure of the solution. It will be shown in a later paper that the results are not due to change in ratio of anions. The second consideration can be ruled out on the basis of data at hand. Between pH 5.0 and pH 8.0 there is a decrease in osmotic pressure calculated as not exceeding 20 per cent. This change is a function of dilution of the phosphate of the acid component by the addition of the alkaline component and therefore varies regularly though not linearly with hydrogen ion concentration. The rate of locomotion, however, remains fairly constant between pH 5.0 and pH 7.5 (the range of maximum change in osmotic pressure) and then drops suddenly at pH 8.0. Furthermore, the difference in osmotic pressure between the dilute and the concentrated solution (curves A and B) at the same hydrogen ion concentration is at least twenty-five times greater than the maximum difference in osmotic pressure in a given series of solutions between pH 5.0 and 8.0. Yet the maximum difference in rate of locomotion in the two salt concentrations (curves A and B) at any given hydrogen ion concentration is less than the variation in the rate of locomotion in the more dilute solution between pH 5.0 and 8.0 (curve A), though the change in osmotic pressure is twenty-five times as great. From this consideration it is concluded that the curves presented describe the relation between rate of locomotion and hydrogen ion concentration and are not appreciably affected by the relatively small changes in osmotic pressure.

The processes involved in the interrelations established will be considered in the third paper in this series.

SUMMARY

1. As the acidity decreases the rate of locomotion of *Amoeba proteus* in a balanced salt solution increases to a

maximum at pH 6.2, then decreases to a median minimum at pH 7.0 after which it increases to a secondary maximum at pH 7.5 and then rapidly decreases to zero at pH 8.0+.

2. The rate of locomotion varies remarkably little over the range of hydrogen ion concentration from pH 5.0 to pH 7.5.

3. The rate of locomotion throughout the entire range of hydrogen ion concentrations tested varies inversely with the salt concentration but the relation between rate of locomotion and hydrogen ion concentration is independent of the salt concentration, except that the difference between maximum and median minimum rates probably decreases with increase in salt concentration.

4. The gel/sol ratio varies directly with the acidity and with the salt concentration, i.e., the higher the hydrogen ion and the salt concentrations, the more plasmagel in relation to plasmasol. This probably holds only for a limited range of concentrations.

5. The rate of locomotion is not directly correlated with the gel/sol ratio.

LITERATURE CITED

- ARMSTRONG, P. B. 1928 The antagonism between acetic acid and the chlorides of sodium, potassium, and calcium as manifested in developing *Fundulus* embryos. *J. Gen. Physiol.*, vol. 11, pp. 515-523.
- BARTH, L. G. 1929 The effect of acids and alkalis on the viscosity of protoplasm. *Protoplasma*, vol. 7, pp. 505-534.
- CHALKLEY, H. W. 1930 On the relation between resistance to heat and the mechanism of death in *Paramoecium*. *Physiol. Zool.*, vol. 3, pp. 425-440.
- CHAMBERS, R. 1921 The effect of experimentally induced changes in consistency on protoplasmic movement. *Proc. Soc. Exp. Biol. and Med.*, vol. 19, pp. 87-88.
- FARR, C. H. 1928 Studies on the growth of root hairs in solutions. IV. The pH-molar-rate relation for collards in calcium chloride. *Am. J. Bot.*, vol. 15, pp. 6-31.
- HOPKINS, D. L. 1926 The effect of hydrogen ion concentration on locomotion and other life processes in *Amoeba proteus*. *Proc. Nat. Acad. Sci.*, vol. 12, pp. 311-315.
- 1929 The effects of substratum, divalent and monovalent cations on locomotion in *Amoeba proteus*. *J. Morph. and Physiol.*, vol. 48, pp. 371-383.

- LEWIS, M. R. 1923 Reversible gelation in living cells. Johns Hopkins Univ. Hosp. Bull., vol. 34, pp. 373-379.
- LILLIE, R. S. 1906 The relation of ions to contractile processes. I. The action of salt solutions on the ciliated epithelium of *Mytilus edulis*. Am. J. Physiol., vol. 17, pp. 89-141.
- LOEB, J. 1911 The role of salts in the preservation of life. Science, N.S., vol. 34, pp. 653-665.
- LOEB, L. 1921 Amoeboid movement, tissue formation, and consistency of protoplasm. Am. J. Physiol., vol. 56, pp. 141-167.
- MAST, S. O. 1929 Mechanics of locomotion in *Amoeba proteus* with special reference to the factors involved in attachment to the substratum. Protoplasma, vol. 8, pp. 344-377.
- MAST, S. O., AND C. L. PROSSER 1932 Effect of temperature, salts, and hydrogen ion concentration on rupture of the plasmagel sheet, rate of locomotion, and gel/sol ratio in *Amoeba proteus*. J. Cell. and Comp. Physiol., vol. 1, pp. 333-354.
- OSTERHOUT, W. J. V. 1912 The permeability of protoplasm to ions and the theory of antagonism. Science, N.S., vol. 35, pp. 112-115.
- PANTIN, C. F. A. 1923 On the physiology of amoeboid movement. I. J. Mar. Biol. Assoc., vol. 13, pp. 24-69.
- 1924 On the physiology of amoeboid movement. II. The effect of temperature. Brit. J. Exp. Biol., vol. 1, pp. 519-537.
- PITTS, R. F. 1932 Constant temperature apparatus adapted for use on the microscope stage. Science, N.S., vol. 76, pp. 626-627.
- 1933 The relation between rate of locomotion and form in *Amoeba proteus*. Biol. Bull., vol. 64, pp. 418-423.
- SCHWITALLA, A. M. 1924 The influence of temperature on the rate of locomotion in *Amoeba*. II. The rate of locomotion of *Amoeba* at different temperatures. J. Morph. and Physiol., vol. 41, pp. 45-62.

THE RELATION BETWEEN INORGANIC SALT CONCENTRATION, HYDROGEN ION CON- CENTRATION AND PHYSIOLOGICAL PROC- ESSES IN AMOEBA PROTEUS

II. RATE OF LOCOMOTION, GEL/SOL RATIO, AND HYDROGEN ION CON- CENTRATION IN SOLUTIONS OF SINGLE SALTS

R. F. PITTS AND S. O. MAST
Zoölogical Laboratory, The Johns Hopkins University

SEVEN FIGURES

(Received for publication October 16, 1933)

INTRODUCTION

In the preceding paper of this series it was demonstrated that in a balanced salt solution simulating the culture medium, the curve expressing the relation between hydrogen ion concentration and rate of locomotion in *Amoeba proteus* is bimodal with maxima at pH 6.2 and 7.5, and a median minimum at pH 7.0, and that the rate of locomotion in this solution is not directly correlated with the gel/sol ratio. Edwards and Forgrave ('23), and Mast and Hulpieu ('30), showed that the rate of locomotion of *Amoeba proteus* in solutions of single salts depends on the kind and the concentration of salt present. Mast and Prosser ('32) confirmed this and they ascertained the gel/sol ratio in relation to the concentration of the salts, but they did not ascertain the relation between rate of locomotion, gel/sol ratio, hydrogen ion concentration, and salt concentration. The following pages concern this relation in sodium, potassium, and calcium salt solutions.

SODIUM SALTS

Observations were made on the effect of hydrogen ion concentration on rate of locomotion and gel/sol ratio in solutions containing sodium ions in four different concentrations, namely, 0.001N, 0.002N, 0.005N, and 0.01N. These solutions consisted of sodium phosphate buffer mixtures ($\text{NaH}_2\text{PO}_4 + \text{NaOH}$). By mixing in different proportions the primary phosphate of a given concentration in reference to sodium with the hydroxide of the same concentration, a series of hydrogen ion concentration was obtained with the same sodium ion concentration throughout.¹ The observations were made as described in the preceding paper. The results obtained are presented in figure 1. For evidence concerning individual variation in the rate of locomotion in *Amoeba proteus* see table 2 in the preceding paper.

Figure 1 shows clearly that the effect of hydrogen ion concentration on the rate of locomotion in sodium salt solutions depends upon the salt concentration. It shows that as the hydrogen ion concentration decreased the rate of locomotion in each sodium concentration used increased gradually to a maximum and then decreased rapidly to zero, but that the maximum shifted from pH 6.8 in sodium concentration of 0.001N to pH 5.3 in sodium concentration of 0.01N, and that the most alkaline solution in which locomotion was observed shifted from pH 8.0 to 6.8. It is especially noteworthy as indicated in figure 1, that at any given hydrogen ion concentration the rate of locomotion decreases as the salt concentration increases, and that the curves deviate more widely as the solutions become more alkaline, indicating that the effects of difference in salt concentration become more pronounced as the solution becomes more alkaline.

By comparing the curves in figure 1 with curves A and B in figure 1 in the preceding paper it will be seen that the results obtained in the four concentrations of the sodium salt solution are markedly different from those obtained in the bal-

¹ At the time these results were obtained only a colorimetric method for hydrogen ion determination was available, the standards ranging from pH 5.3 to pH 8.0.

anced salt solution in that in the former there is no double optimum and there is a marked decrease in the rate of locomotion in hydrogen ion concentration lower than pH 7.0. These results indicate that the bimodal curves obtained by Hopkins,

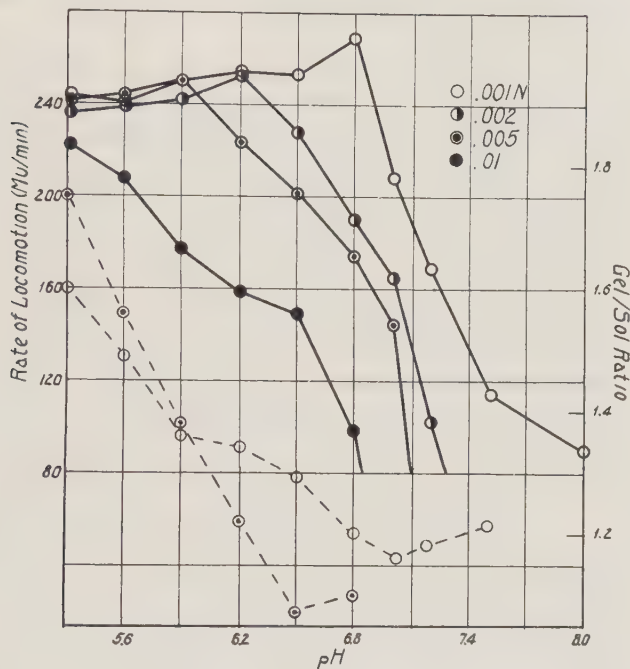


Fig. 1 The relation between rate of locomotion, gel/sol ratio, hydrogen ion concentration and sodium ion concentration. Each of the curves represents a series of experiments conducted at constant sodium ion concentration. The four solid curves are based on the measurement of rate of locomotion of an average of 14.8 different specimens for an average of 88.1 minutes in each hydrogen ion concentration tested. The two broken curves are based on the measurement of the gel/sol ratio in an average of 19 different specimens in each hydrogen ion concentration tested.

and Mast and Prosser, and those presented in the preceding paper are due either to the combination of different salts used or to the concentration of the salts present.

The gel/sol ratio of the amoebae used in ascertaining the rate of locomotion in the observations considered above was measured as described in the preceding paper. Figure 1 in-

icates that as the hydrogen ion concentration decreased the gel/sol ratio rapidly decreased to a minimum, and then increased slightly. The following discussion indicates, however, that while the gel/sol ratios indicated in the first part of the curves are doubtless nearly correct, those indicated in the second part are not.

It is customary in ascertaining the gel/sol ratio, to measure the outer limits of the animal and the limits of the plasmasol and to subtract the latter from the former to obtain the thickness of the plasmagel, the hyaline layer which is included as gel being normally in balanced solutions not sufficient to cause any considerable error. But it was noted that as the solutions of sodium salts became less acid this hyaline layer increased greatly in thickness and that it was impossible to distinguish definitely the outer border of the plasmagel and thus to make correction for the hyaline layer. The results obtained in the more alkaline solutions are therefore too large. It is consequently probable that there is actually no increase in the gel/sol ratio as the alkaline limit of the range is approached. If this is true it will be seen by comparing figure 1 in this paper with figure 1 A₁ and B₁ in the preceding paper that although the magnitude of the gel/sol ratios differs, the relation between gel/sol ratio and hydrogen ion concentration in the 0.001N and 0.005N sodium solutions and in the balanced salt solutions is qualitatively similar in that the amount of plasmasol increases at the expense of the plasmagel as the hydrogen ion concentration decreases. The curves in figure 1 show that in the more acid range (pH 5.3 to 5.9) the gel/sol ratio was higher in the more concentrated solution, i.e., that in this range the relation between gel/sol ratio and salt concentration was the same for the sodium salt solution as it was for the balanced salt solutions, but that in the less acid range (pH 5.9 to 6.5) the reverse obtained.

POTASSIUM SALTS

The observations on amoebae in solutions containing potassium as the only metallic cation were made with potassium

phosphate buffers ($\text{KH}_2\text{PO}_4 + \text{KOH}$) made up in the same way as the sodium phosphate buffers used in the preceding experiments with the exception that in the extreme acid range primary potassium phosphate containing phosphoric acid in equimolar concentration was added to primary potassium phos-

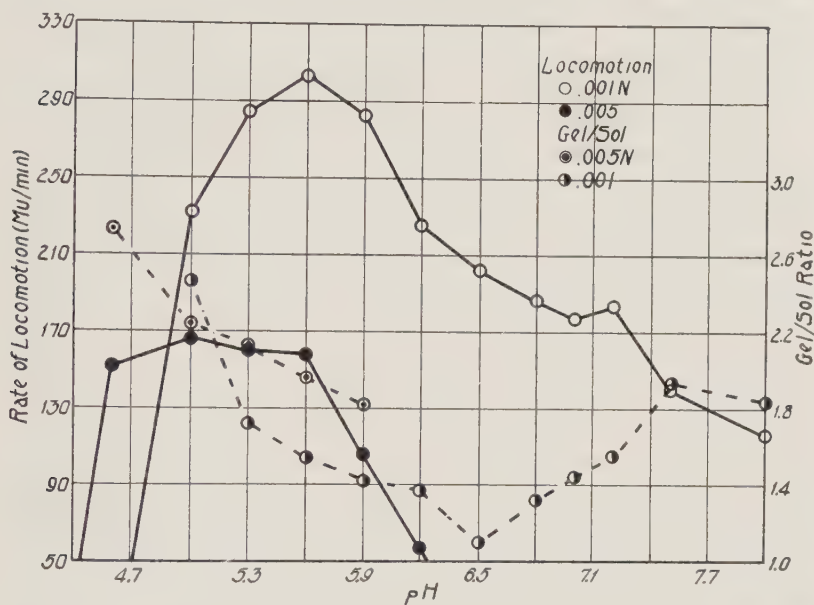


Fig. 2 The relation between rate of locomotion, gel/sol ratio, hydrogen ion concentration, and potassium ion concentration. Curve $\circ$, mean rate of locomotion in 0.001N based on measurements on an average of 22.2 different specimens for a total average of 111.4 minutes in each hydrogen ion concentration tested; curve $\bullet$, mean rate of locomotion in 0.005N, based on measurements on an average of 12 different specimens for a total average of 69.3 minutes in each hydrogen ion concentration tested; curve $\odot$, mean gel/sol ratio in 0.001N based on measurements on an average of 18.7 different specimens in each hydrogen ion concentration tested; curve $\ominus$, mean gel/sol ratio in 0.005N based on measurements on an average of 12.2 specimens in each hydrogen ion concentration tested.

phate in varying amounts ($\text{KH}_2\text{PO}_4 + \text{KH}_2\text{PO}_4, \text{H}_3\text{PO}_4$). However, the concentration of potassium was the same in all the mixtures for it was present in the same concentration in all the solutions used. Experiments were performed with solutions containing potassium ions in three different concentrations, namely, 0.001N, 0.005N, and 0.01N. The results obtained are presented in figure 2.

It will be seen by comparing figure 2 with figure 1, that the results obtained with potassium are in a general way similar to those obtained with sodium. These figures show that there was for both salt solutions a single optimum for locomotion which lay on the acid side of neutrality, that the position of the optimum was more acid in potassium solutions than in sodium solutions of the same concentration, that low rates of locomotion in the alkaline range were noted in both sodium and potassium solutions, and that the most alkaline solution in which locomotion occurred was in both shifted more and more to the acid side with increase in salt concentration. It will also be seen that the optimum hydrogen ion concentration in both shifted toward the acid range with increase in salt concentration, but that the extent of the shift was less in potassium solutions than in sodium solutions, the shift in sodium solutions having been from pH 6.8 to pH 5.9, with increase in concentration from 0.001N to 0.005N, while in potassium it was only from pH 5.6 to pH 5.0 for corresponding change in concentration. Furthermore it will be seen that the most acid solution in which locomotion occurred for potassium solutions was increased with increased salt concentration; that is, the more concentrated the solution, the higher the hydrogen ion concentration required to prevent attachment.

Thus in general, the relation between rate of locomotion, hydrogen ion concentration, and salt concentration appears to be essentially the same for solutions of potassium and sodium salts. The only marked difference found is that in potassium solutions 0.010N, no locomotion was observed in any of the hydrogen ion concentrations tested (pH 4.2 to pH 8.0). while in the sodium solution 0.010N, locomotion was observed in hydrogen ion concentrations between pH 5.3 and pH 6.8.

Figure 2 shows that in potassium solutions as in sodium solutions the gel/sol ratio decreases as the hydrogen ion concentration decreases. The marked rise in gel/sol ratio in the 0.001N potassium solution in the alkaline range is probably due to increase in the hyaline layer as explained above.

CALCIUM SALTS

The observations on amoebae in solutions containing calcium as the only metallic cation were made with calcium phosphate buffers ($\text{CaH}_4(\text{PO}_4)_2 + \text{Ca}(\text{OH})_2$), with dilute phosphate buffers ($\text{CaH}_4(\text{PO}_4)_2 + \text{CaCl}_2 + \text{Ca}(\text{OH})_2, \text{CaCl}_2$), and without buffering ($\text{CaCl}_2 + \text{Ca}(\text{OH})_2, \text{CaCl}_2$) depending on the hydrogen ion concentration and the salt concentration desired. Calcium becomes more and more insoluble in phosphate solution as the hydrogen ion concentration decreases. It was therefore found to be necessary to reduce the phosphate in preparing solutions with an hydrogen ion concentration of pH 6.8 and 7.0 in calcium 0.005M, and to omit it in preparing solutions with hydrogen ion concentrations of pH 8.0 in calcium 0.001M and of pH 7.5 and 8.0 in calcium 0.005M. This was done by substituting CaCl_2 in part or entirely for $\text{CaH}_4(\text{PO}_4)_2$ as indicated above.

The results obtained are presented in figure 3. By referring to this figure, it will be seen that in solutions containing calcium 0.001M, the rate of locomotion was practically independent of hydrogen ion concentration over the entire range studied, but that in solutions containing calcium 0.005M, as the hydrogen ion concentration decreased the rate of locomotion increased rapidly at first, and then more gradually to a maximum at pH 7.5, after which it decreased slightly.

On comparing figure 3 with figures 1 and 2, the following is clearly evident. The effect of hydrogen ion concentration on rate of locomotion in solutions of calcium salts differs greatly from that in solutions of either sodium or potassium salts. Indeed the effect appears to be similar in only a few respects. In the calcium solution just as in the sodium and the potassium solutions there is but one optimum hydrogen ion concentration in reference to rate of locomotion. The rate of locomotion in all three decreased throughout the range of hydrogen ion concentration studied, with increase in salt concentration. This is in accord with the results of Edwards and Forgrave ('23) and Mast and Prosser ('32). In all three solutions as in the solution containing a mixture of salts, the rate of loco-

motion suddenly dropped to zero at the acid end of the range. This seems to be conditioned merely by the failure to attach.

Some striking differences are evident which indicate in the main that the action of calcium differs qualitatively from that of either sodium or potassium. The variation in the rate of locomotion over the range of hydrogen ion concentrations be-

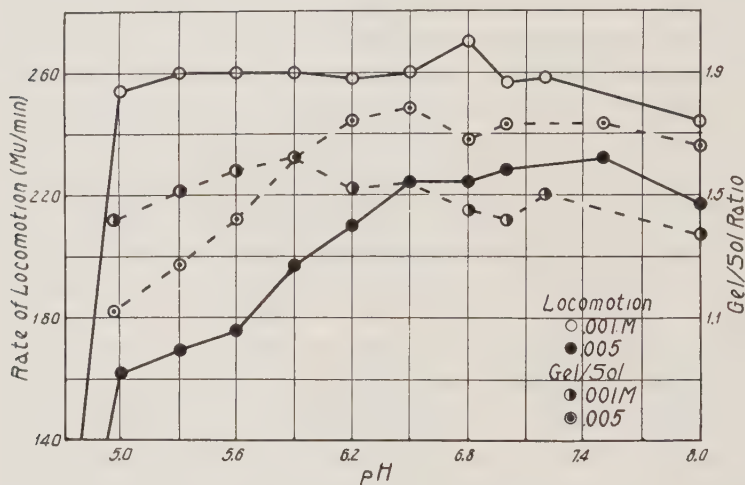


Fig. 3 The relation between rate of locomotion, gel/sol ratio, hydrogen ion concentration and calcium ion concentration. Curve $\circ$, mean rate of locomotion in 0.001M based on measurements on an average of 22.1 different specimens for 111.6 minutes in each hydrogen ion concentration tested; curve $\bullet$, mean rate of locomotion in 0.005M based on an average of 18.7 different specimens for a total average of 96.6 minutes in each hydrogen ion concentration tested; curve $\bigcirc$, mean gel/sol ratio in 0.001M based on measurements on an average of 21.3 different specimens in each hydrogen ion concentration tested; curve $\odot$, mean gel/sol ratio in 0.005M based on measurements on a total average of 14.6 different specimens in each hydrogen ion concentration tested.

tween pH 5.0 and 8.0 was very much smaller in solutions containing calcium than in those containing either sodium or potassium. This difference is particularly striking in the alkaline range where it will be seen that there was only a slight decrease in the former and a very great decrease in both of the latter. The optimum hydrogen ion concentration, it will be seen, shifted in the calcium solutions from pH 6.8 in 0.001M to pH 7.5 in 0.005M, i.e., with increase in salt

concentration it shifted in precisely the opposite direction from that which obtained for sodium and potassium respectively. Moreover, the difference in the rate of locomotion in different concentrations is much greater in the acid range than it is in the alkaline range for calcium and precisely the opposite for sodium and potassium, respectively. Furthermore, the gradual increase in rate in calcium 0.005M from zero in acidity above pH 5.0 to a maximum at pH 7.5 finds no counterpart in any of the other solutions used.

By comparing the curves in figures 1, 2, and 3 it will be seen that the maximum average rate of locomotion observed in the three salts considered was highest for potassium (303 μ per minute) and only slightly lower for sodium and calcium (269 and 270 μ per minute respectively), but that the rate was high over a much wider range in the calcium solutions than it was in either the potassium or the sodium solutions.

By referring to figure 3 it can be seen that the gel/sol ratio in calcium 0.001M remained fairly constant throughout the range of hydrogen ion concentration from pH 5.0 to 8.0 paralleling in general the rate of locomotion over this range. This is in marked contrast with the results obtained in either the balanced salt solution or in solutions of sodium or potassium alone for in these solutions there was definite decrease in gel/sol ratio with increased alkalinity of the solution. The difference is even more marked in calcium 0.005M in which there was considerable increase in gel/sol ratio as the hydrogen ion concentration decreased from pH 5.0 to 6.2 after which there was practically no change. It can also be seen that in the sodium solutions the gel/sol ratio was higher in the stronger solution than in the weaker in the more acid range (pH 5.0 to 5.9) and the reverse in the less acid range (pH 5.9 to 6.5), that it was precisely the opposite in the calcium solutions, being lower in the stronger solution in the more acid (pH 5.0 to 5.6) and higher in the weaker solution in the less acid range (pH 5.6 to 8.0), and that in the potassium solutions the results are indefinite owing, doubtless, to the high toxicity of this substance.

Thus it seems that in solutions containing calcium as the only metallic cation, the relation between rate of locomotion, gel/sol ratio, and hydrogen ion concentration is in many respects inverse to that for solutions containing sodium or potassium as the only metallic cation. But that there is no evidence of a specific correlation between gel/sol ratio and rate of locomotion in any of the solutions tested.

These results support the conclusions reached by Mast and Prosser ('32, p. 351) that, "the statement frequently found in the literature that divalent cation salts gelate and monovalent solate is misleading," and that "probably all salts tend to gelate if the concentration is increased and solate if the concentration is decreased." They show that the relation between concentration of both mono- and divalent cations and their action on the consistency of protoplasm is specifically correlated with the hydrogen ion concentration.

THE EFFECT OF ANIONS ON RATE OF LOCOMOTION IN AMOEBA PROTEUS

The question as to whether the variation in rate of locomotion observed in the preceding sections is due to the change in hydrogen ion concentration or to the parallel change in the kind of anions and their ratio was investigated as follows. The rate of locomotion was ascertained in a series of hydrogen ion concentrations in two solutions, in both of which the sodium concentration was kept constant at 0.005N. In one solution there were only phosphate and hydroxyl anions, while in the other there were phosphate, chloride, and hydroxyl anions. The former solution was prepared as described above; the latter was prepared by mixing in various proportions as acid component containing NaCl 0.004N and NaH_2PO_4 0.001N, with a basic component containing NaCl 0.004N and NaOH 0.001N. Since the total concentration of sodium was 0.005N in both components, no change in sodium ion concentration occurred on mixing. In the latter solution the total concentration of phosphate, the ratio of primary to secondary phosphate, and the ratios of chloride to primary

and to secondary phosphate respectively changed with change in hydrogen ion concentration. In the former solution only the total concentration of phosphate and the ratio of primary to secondary phosphate changed. Thus if anions affect the rate of locomotion there should be a difference in the rates observed at corresponding hydrogen ion concentrations in the two solutions. The results obtained are presented in figure 4.

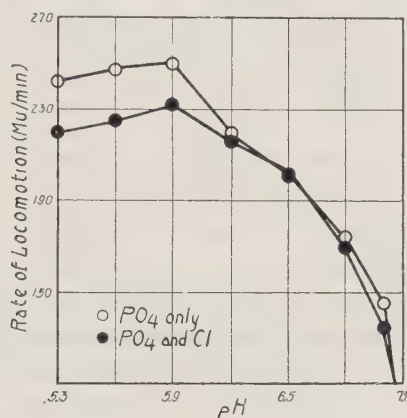


Fig. 4 The effects of anions on rate of locomotion in solutions containing constant sodium ion concentration. Curve ●, mean rates of locomotion in solution containing chloride and phosphate based on measurements on an average of 17.9 different specimens for a total average of 90.9 minutes in each hydrogen ion concentration tested. The curve ○ for the solution containing only phosphate is transferred from figure 1.

It is evident that the two curves in their form are remarkably similar in form, especially in the range from pH 6.2 to 7.0 where they are almost identical, indicating that over this range the effects on rate of locomotion in the two solutions were practically the same. Between pH 5.3 and 5.9 the curves though paralleling each other closely indicate rates of locomotion somewhat lower for the chloride phosphate solution than for the solution containing phosphate alone. However these observations were made on animals from different cultures. The lack of absolute agreement is therefore probably due to difference in the amoebae rather than to differ-

ence in effect of the two solutions. It is concluded from these observations that substitution of chloride for phosphate does not affect the rate of locomotion in *Amoeba proteus*.

The inference is logical then that alteration of the ratio of primary and secondary phosphate does not in itself affect the rate of locomotion. If this inference is valid then the variation in rate of locomotion considered in the preceding and the following sections may be considered as being independent of changes in the kind or concentration of the anions, at least as far as primary and secondary phosphate and chloride are concerned.

THE EFFECT ON AMOEBA PROTEUS OF PROLONGED EXPOSURE IN VARIOUS SOLUTIONS

The marked decrease in the rate of locomotion in the alkaline and the extreme acid ranges of the single salt solutions of sodium and potassium is striking, and preliminary observations in the more concentrated salt solutions seem to indicate that this is correlated with an effect that continues for a considerable time. The following experiments concern this problem.

Amoebae were washed in redistilled water, selected, and transferred to 0.005N solutions of sodium, potassium, and calcium phosphate buffer solutions at hydrogen ion concentrations ranging from pH 4.2 to 8.0, as previously described. These solutions were identical with those used in the preceding experiments. The dishes containing the amoebae were then sealed with vaseline and set aside for a period of 4 hours. At the end of this time the covers were removed, the solutions withdrawn with a pipette, and Chalkley solution² (pH 6.5) poured on and removed with a pipette three times. Then after 1 hour in Chalkley solution, the rate of locomotion of the amoebae was ascertained as described above. As control experiments amoebae taken from the same cultures as those which had been put into the buffer solutions were washed in redistilled water; then within 10 minutes some which had be-

² For composition of Chalkley solution see the preceding paper, p. 451.

come radiate in form were selected, transferred to Chalkley solution (pH 6.5) and left an hour, after which the rate of locomotion was ascertained; others were left in redistilled water 4 hours, then transferred to Chalkley solution and left for 1 hour, after which the rate of locomotion was ascertained.

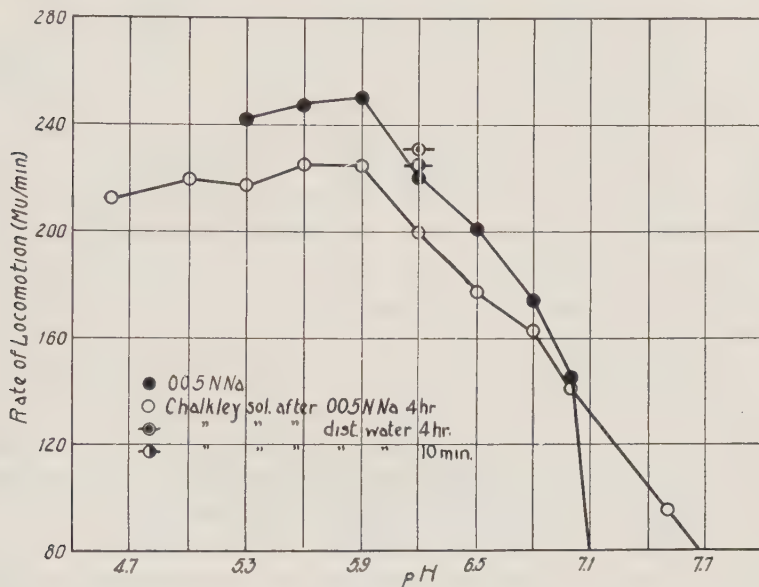


Fig. 5 The effect on rate of locomotion in Chalkley solution, pH 6.5, of prolonged exposure to sodium solutions 0.005N varying in hydrogen ion concentration from pH 4.6 to 8.0. Curve ○, mean rates of locomotion in Chalkley solution, pH 6.5, based on the measurement on an average of 10.1 different specimens for a total average of 48.5 minutes in each hydrogen ion concentration tested. ○ and ● (two controls), mean rates of locomotion based on measurements of averages of, respectively, 11 and 10 different specimens measured for a total of 52 and 49 minutes, respectively. The curve for locomotion in 0.005N sodium is transferred from figure 1.

By referring to figure 5 the following will be seen: The rate of locomotion in the amoebae which had been in pure water 4 hours and then in Chalkley solution 1 hour was essentially the same as it was in those which had been in pure water only a few minutes and then in Chalkley solution 1 hour, that is, an average of 231μ per minute for the former and 225μ per

minute for the latter. This shows that exposure to pure water as long as 4 hours does not seriously injure *Amoeba proteus*, the effect being entirely eliminated by the action of the Chalkley solution for 1 hour.

The rate of locomotion in the amoebae which has been in solutions of sodium 4 hours and then in Chalkley solution 1 hour was, in all tests in which the hydrogen ion concentration of the sodium solution was pH 5.9 or higher, nearly the same as it was in the controls, i. e., in those which had not been subjected to sodium, but it was lower in all in which the hydrogen ion concentration of this solution was lower than pH 5.9; and the lower the hydrogen ion concentration the greater the difference in rate. At the lowest hydrogen ion concentration tested (pH 8.0) the rate was practically zero. Only a few specimens were active at all and all showed signs of disintegration.

By comparing the two curves in figure 5 it will be seen that the effect of 4 hours in solutions of sodium ranging in hydrogen ion concentration from pH 5.9 to pH 4.5 (if there actually is any) is eliminated after 1 hour in Chalkley solution, but that the effect of 4 hours in solutions of sodium which are more alkaline than pH 5.9 is changed only slightly if at all during 1 hour in Chalkley solution. This indicates that in solutions of sodium which are alkaline or only slightly acid the amoebae are definitely injured and that the extent of this injury varies inversely with acidity.

The results obtained on the effect of prolonged exposure to potassium are presented in figure 6. This figure shows that the rate of locomotion in amoebae which had been in solutions of potassium 4 hours and then in Chalkley solution 1 hour was, in the tests in which the hydrogen ion concentration of the potassium solution was pH 5.3, 5.0, and 4.7, nearly normal, that is, nearly the same as it was in the amoebae which had been only a short time in pure water and then 1 hour in Chalkley solution. But that in the tests in which the potassium solution was more acid than pH 4.7 or more alkaline than pH 5.3, the rate was markedly lower, especially in the alka-

line range. It shows, however, that practically throughout the entire range the rate was much higher than it was in amoebae which were in potassium solution when the rate was measured (curve ●) even if they had been in this solution only a short time.

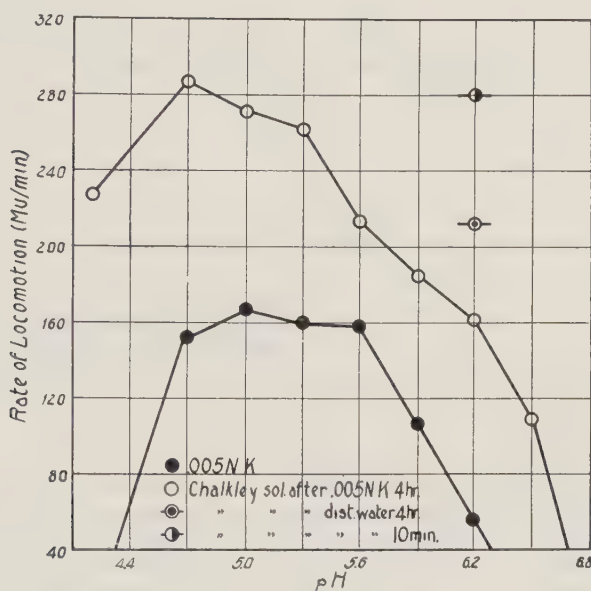


Fig. 6 The effect on rate of locomotion in Chalkley solution, pH 6.5, of prolonged exposure to potassium solutions 0.005N varying in hydrogen ion concentration from pH 4.2 to 7.0. Curve O, mean rates of locomotion in Chalkley solution pH 6.5, based on the measurement on an average of 16.5 different specimens for a total average of 86 minutes in each hydrogen ion concentration tested. ● and ◐ (two controls), mean rates of 17 and 16 different specimens measured for a total of 89 and 82 minutes, respectively. The curve for locomotion in 0.005N potassium is transferred from figure 3.

This shows that the action of potassium in all hydrogen ion concentrations tested interferes with locomotion, that the effect is drastic in the more alkaline solutions (pH 5.9 to 8.0) and recovery slow, but that recovery is fairly rapid in the more acid solutions (pH 5.3 to 4.7).

It is probable that a part of the retarding effect of potassium on locomotion is due to reduction in adhesiveness of the

surface and consequent interference with attachment to the substratum.

The results obtained on the effect of prolonged exposure to calcium are presented in figure 7. This figure shows that the rate of locomotion in amoebae which have been in calcium

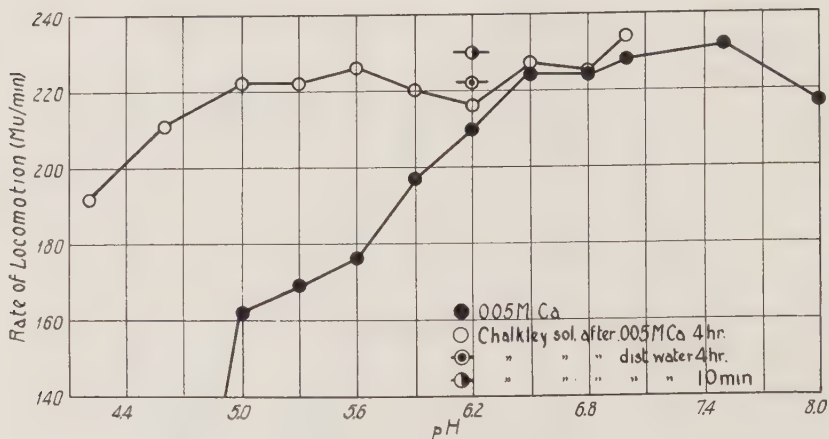


Fig. 7 The effect on rate of locomotion in Chalkley solution, pH 6.5, of prolonged exposure to calcium solutions 0.005N varying in hydrogen ion concentration from pH 4.2 to 7.0. Curve O, mean rates of locomotion in Chalkley solution, pH 6.5, based on the measurement of an average of 14.9 different specimens for a total average of 72.6 minutes in each hydrogen ion concentration tested. ◐ and ● (two controls), mean rates of 15 different specimens measured for a total of 75 minutes. The curve for locomotion in 0.005M calcium solutions is transferred from figure 4.

solutions 4 hours and then in Chalkley solution 1 hour was nearly normal in all tests in which the hydrogen ion concentration of the calcium solution was pH 5.0 or lower,³ but that it was considerably lower in those in which the calcium solutions were more acid than pH 5.0. By comparing these results with those obtained in observations on the rate of locomotion of amoebae in calcium solution (curve ●) it will be

³ Owing to the slight solubility of calcium phosphate in solutions more alkaline than pH 7.0, it was necessary to use unbuffered solutions to obtain hydrogen ion concentrations lower than pH 7. However, the hydrogen ion concentration of unbuffered solutions, even though in vaseline sealed dishes, changed so much during the 4-hour period of the experiment that it rendered the results obtained valueless. They are therefore not presented.

seen that the rate of locomotion in the calcium solutions was in all tests in which these solutions were more alkaline than pH 6.0, nearly the same as the rate in amoebae which had been in calcium solutions 4 hours and then in Chalkley solution 1 hour, but that it was much lower in those which were more acid. The results presented consequently show that calcium interferes with locomotion in solutions which are more acid than pH 6.0, but not in solutions which are less acid, and they show that transfer from the calcium solutions to favorable balanced salt solutions results in rapid recovery.

DISCUSSION

Pantin ('26) states that locomotion in a marine limax amoeba does not occur in single isotonic salt solutions of sodium, potassium, or calcium, i.e., that locomotion occurs in sodium or potassium solutions only in the presence of calcium, and in solutions of calcium only in the presence of one of the former. Hopkins ('29) asserts that calcium or strontium is necessary for locomotion of *Amoeba proteus* in solutions of sodium or potassium salts. Edwards and Forgrave ('23) contend, however, that *Amoeba proteus* can move in solutions containing only sodium or potassium salts, and Mast ('29), Mast and Hulpieu ('30), and Mast and Prosser ('32) maintain that it can move in solutions containing only sodium, potassium, calcium, or magnesium. That is Pantin and Hopkins hold that a bivalent cation salt is necessary for movement of *Amoeba* in solutions of monovalent cation salts and vice versa, and the rest of the investigators hold that it is not necessary. The results presented above obviously support the latter view. How then can the results obtained by Pantin and Hopkins be explained?

Pantin, as previously stated, studied a marine limax amoeba and used solutions of various salts isotonic with sea water. Figure 1 of the present paper indicates that if the sodium concentration had been increased until it was isotonic with sea water the hydrogen ion concentration required to permit locomotion in *Amoeba proteus* would have been so high that

it would have prevented locomotion if it did not actually kill the amoebae, i.e., it would have been higher than that which Pantin found inhibited locomotion in marine amoebae. The results presented in figure 3 support this contention for they show that in potassium 0.01N no locomotion was obtained in hydrogen ion concentrations between pH 4.2 and 8.0. This seems to indicate that amoebae which require concentrations of salts isotonic with sea water will not move in solutions containing but one salt. Hopkins maintains that no locomotion occurs in *Amoeba proteus* in a 0.00678N solution of a sodium or potassium salt, pH 6.6. By referring to figures 1 and 3 it can be seen that this contention is confirmed in reference to potassium but not in reference to sodium, for the curves clearly indicate that locomotion would occur in sodium solution 0.00678N, pH 6.6, but that the rate would be rather low.

The processes involved in the interaction between different salts in relation to gel/sol ratio and rate of locomotion in *Amoeba proteus* will be considered in the next paper in this series.

SUMMARY

1. As the hydrogen ion concentration decreases the rate of locomotion of *Amoeba proteus* in solutions containing only sodium, potassium, or calcium salts in various concentrations increases from zero to a maximum and then decreases to zero, that is, there is but one maximum. The relation between rate of locomotion and hydrogen ion concentration in solutions containing but one salt therefore differs radically from that in solutions containing several salts in balanced proportions, in which there usually, if not always, are two maximum rates with a median minimum rate at neutrality.

2. In solutions containing only sodium salts the rate of locomotion in general varies inversely with the concentration and as the concentration increases over the range tested the optimum acidity shifts from pH 6.5 to pH 5.0 and the maximum alkalinity permitting locomotion from pH 8.0 to pH 6.9.

3. In solutions containing only potassium salts the relation between concentration and rate of locomotion is similar to that in solutions containing only sodium salts.

4. In solutions containing only calcium salts the rate of locomotion varies inversely with salt concentration as it does in sodium and potassium solutions but the optimum shifts in the opposite direction and the maximum alkalinity permitting locomotion is much higher.

5. The maximum rate of locomotion observed is highest for potassium and only slightly lower for sodium and calcium solutions, but the rate is high over a much wider range in calcium solutions than it is in either potassium or sodium salt solutions.

6. The rate of locomotion is probably independent of the kind and the concentration of anions.

7. Exposure of amoebae to solutions of single salts for a period of 4 hours causes injury which is not eliminated in a period of 1 hour in a favorable solution. The extent of this injury depends on the hydrogen ion concentration and the specific salt to which the amoebae were exposed. In solutions of sodium or potassium salts, the injury is greatest in the more alkaline solutions; in solutions of calcium salts it is greatest in the more acid solutions. In all three salts there is evidence of some injury in extremely acid solutions. The extent of the injury in any one of the three single salt solutions is thus roughly inversely proportional to the rate of locomotion in that solution.

8. In sodium or potassium solutions the gel/sol ratio (qualitative measure of viscosity) decreases as the hydrogen ion concentration decreases. In calcium solutions it increases in the more acid range (pH 5.0 to 5.9), then remains constant or decreases slightly.

9. In sodium solutions, as the salt concentration increases, the gel/sol ratio increases in the more acid range (pH 5.3 to 5.9) and decreases in the less acid range (pH 5.9 to 6.8). In calcium solutions the reverse obtains. The potassium solutions used are so toxic that the results have little if any comparative value.

10. There is no specific correlation between gel/sol ratio and rate of locomotion in any of the salt solutions tested.

LITERATURE CITED

- EDWARDS, J. G., AND H. S. FORGRAVE 1923 The rate of locomotion of amoeba in alkali chlorides. *J. H. U. Hosp. Bull.*, vol. 34, pp. 387-389.
- HOPKINS, D. L. 1929 The effects of substratum, divalent and monovalent cations on locomotion in *Amoeba proteus*. *J. Morph. and Physiol.*, vol. 48, pp. 371-383.
- MAST, S. O. 1929 Mechanics of locomotion in *Amoeba proteus*, with special reference to the factors involved in attachment to the substratum. *Protoplasma*, vol. 8, pp. 344-377.
- MAST, S. O., AND H. R. HULPIEU 1930 Variation in the response to light in *Amoeba proteus* with special reference to effect of salts and hydrogen ion concentration. *Protoplasma*, vol. 11, pp. 312-431.
- MAST, S. O., AND C. L. PROSSER 1932 Effect of temperature, salts, and hydrogen ion concentration on rupture of the plasmagel sheet, Rate of locomotion, and gel/sol ratio in *Amoeba proteus*. *J. Comp. and Cell. Physiol.*, vol. 1, pp. 333-354.
- PANTIN, C. F. A. 1926 On the physiology of amoeboid movement. III and IV. The action of calcium and magnesium. *Brit. J. Exp. Biol.*, vol. 3, pp. 275-311.
- PITTS, R. F. 1933 The relation between rate of locomotion and form in *Amoeba proteus*. *Biol. Bull.*, vol. 64, pp. 418-423.
- PITTS, R. F., AND S. O. MAST, 1933 The relation between inorganic salt concentration, hydrogen ion concentration and physiological processes in *Amoeba proteus*. I. Rate of locomotion, gel/sol ratio, and hydrogen ion concentration in balanced salt solutions. *J. Cell. and Comp. Physiol.*, vol. 3, pp. 449-462.

THE RELATION BETWEEN INORGANIC SALT CON- CENTRATION, HYDROGEN ION CONCENTRATION AND PHYSIOLOGICAL PROCESSES IN AMOEBA PROTEUS

III. THE INTERACTION BETWEEN SALTS (ANTAGONISM) IN RELATION TO HYDROGEN ION CONCENTRATION AND SALT CONCENTRATION

R. F. PITTS AND S. O. MAST

Zoölogical Laboratory, The Johns Hopkins University

SEVEN FIGURES

(Received for publication January 31, 1934)

INTRODUCTION

Since the discovery by Ringer (1882) of antagonism between calcium and sodium or potassium salts in their action on the frog's heart, the problem has been intensively investigated in reference to various physiological processes in many organisms. There have been, however, no extensive observations made on the relation between hydrogen ion concentration, salt concentration and antagonism between different salts. The following experiments concern primarily this relation.

THE RELATION BETWEEN HYDROGEN ION CONCENTRATION AND ANTAGONISM BETWEEN SALTS

Sodium and calcium

Observations were made on the rate of locomotion and the gel/sol ratio in *Amoeba proteus* in solutions containing sodium and calcium in hydrogen ion concentrations varying from pH 4.2 to 8.0. The solutions used were prepared by making two stocks, one containing primary sodium phosphate 0.005N and

calcium chloride 0.001M, the other containing sodium hydroxide 0.005N and calcium chloride 0.001M, and mixing the two stocks in varying proportion to obtain solutions of the desired hydrogen ion concentration. All of the solutions

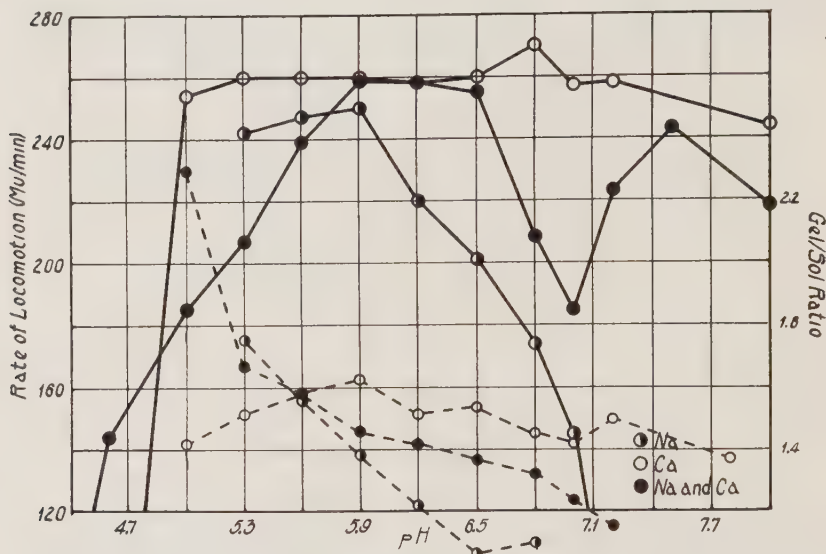


Fig.1 Antagonism between 0.005N sodium and 0.001M calcium. Solid line, rate of locomotion; broken line, gel/sol ratio. The solid curve for the solution containing sodium and calcium is based on the measurement of the rate of locomotion on an average of 14.75 specimens for a total average of 89.9 minutes in each hydrogen ion concentration tested. The solid curves for sodium and calcium, respectively, were transferred from the preceding paper, figures 1 and 3. The broken curve for the solution containing sodium and calcium is based on the measurement of an average of 19.8 different specimens in each hydrogen-ion concentration tested. The broken curves for sodium and calcium were transferred from the preceding paper, figures 1 and 3.

obviously contained the same concentration of sodium and calcium. The observations were made as described in the preceding papers. The results obtained are presented in figure 1. For details concerning individual variation in the rate of locomotion of *Amoeba proteus* see table 2 in the first paper in this series.

Figure 1, curve ●, shows that as the hydrogen ion concentration of the solutions containing sodium and calcium salts decreased, the rate of locomotion increased rapidly to a maximum at about pH 6.2, then decreased sharply to a median minimum at pH 7.0, after which it increased to a secondary maximum at pH 7.5, then decreased again. It also shows that in solutions more alkaline than pH 6.2 the rate for the mixture of calcium and sodium was between the rate for calcium alone (curve ○) and the rate for sodium alone (curve ●).

This indicates that if the solution is more alkaline than pH 6.2 the addition of calcium to sodium solutions causes increase and the addition of sodium to calcium solutions decrease in rate of locomotion, and that increase in hydrogen ion concentration over this range caused very marked increase in rate of locomotion in sodium solutions, but only slight increase if any in calcium solutions. It is consequently evident that addition of calcium to sodium solutions has the same effect as increasing the hydrogen ion concentration and addition of sodium to calcium solution the same effect as decreasing the hydrogen ion concentration. However, examination of the curves in figure 1 shows at once that the magnitude of the effect of changes in the hydrogen ion concentration is not uniform over the entire range considered. For example, it shows that the addition of calcium to sodium solutions has relatively very much greater effect at pH 7.5 than it has at pH 7.0. The cause of this is not obvious.

The figure shows also that in hydrogen ion concentrations higher than pH 5.9, addition of either calcium to sodium solution or sodium to calcium solution causes very marked decrease in rate of locomotion; and that increase in hydrogen ion concentration from pH 5.9 to 5.3 causes only very slight decrease in rate of locomotion in sodium solution and no change in rate of locomotion in calcium solution. This indicates that over this range the effect of increase in hydrogen ion concentration on rate of locomotion in calcium solution and sodium solution, respectively, is the same in direction, in

so far as there is any effect, but that it is not nearly so great as the effect of addition of calcium to sodium or sodium to calcium salt solution.

The conclusion is drawn that calcium and sodium are antagonistic in their effects on rate of locomotion in hydrogen ion concentrations between pH 6.2 and 8.0, and the opposite in hydrogen ion concentrations between pH 6.2 and 4.6, and that the relative extent of the interaction varies greatly with the hydrogen ion concentration.

Figure 1 shows that as the hydrogen ion concentration of the mixture of sodium and calcium decreased the gel/sol ratio decreased, at first rapidly and then more slowly and steadily, and that the gel/sol ratio in the mixture was intermediate for the most part of the range between that in the sodium and the calcium solutions, respectively, though more closely approximating that in sodium so far as the form of the curve is concerned. Thus antagonism between sodium and calcium as concerns gel/sol ratio is not as evident as antagonism in relation to rate of locomotion, for the relation between gel/sol ratio and hydrogen ion concentration in the sodium solution and in the mixture are very similar though the gel/sol ratios differ somewhat in magnitude. It is evident, however, that these relations for both solutions are different from that observed in the calcium solution.

Potassium and calcium

Observations were made on the relation between hydrogen ion concentration, rate of locomotion, and gel/sol ratio in solutions containing potassium 0.005N and calcium 0.001M in hydrogen ion concentrations varying from pH 4.2 to 8.0. The solutions used were the same as those used in the preceding experiments except that potassium phosphate and hydroxide replaced the corresponding sodium salts. The methods of observation were the same. The results obtained are presented in figure 2.

By comparing figure 2 with figure 1 it can be seen that the results obtained with mixtures of potassium and calcium

are similar to those obtained with mixtures of sodium and calcium, the only striking difference being that the rate of locomotion between pH 6.2 and 4.6¹ changed very much more gradually in the potassium calcium mixture than in the sodium calcium mixture. By comparing the rates of locomotion at corresponding hydrogen ion concentrations in the potassium

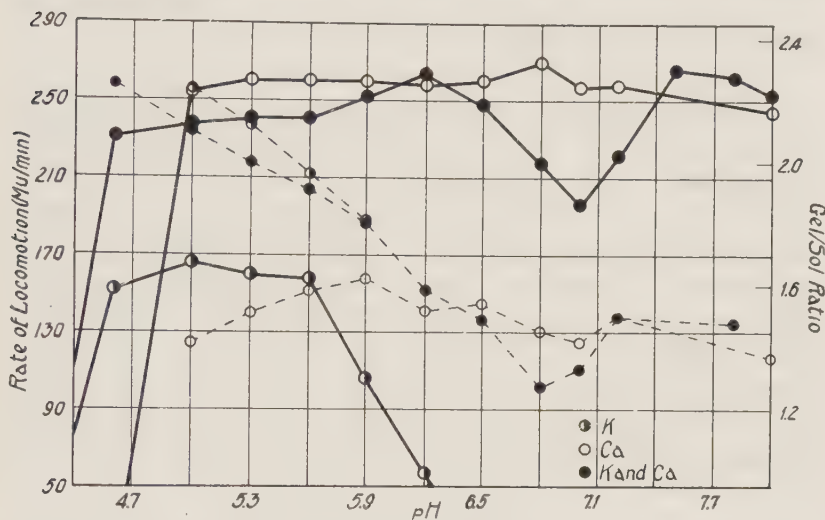


Fig. 2 Antagonism between 0.005N potassium and 0.001M calcium. Solid line, rate of locomotion; broken line, gel/sol ratio. The solid curve for the solution containing potassium and calcium is based on the measurement of the rate of locomotion on an average of 20.5 specimens for a total average of 104.6 minutes in each hydrogen ion concentration tested. The broken curves for potassium and calcium, respectively, were transferred from the preceding paper, figures 2 and 3. The broken curve for the solution containing potassium and calcium is based on the measurement of an average of twenty different specimens in each hydrogen ion concentration tested. The broken curves for potassium and calcium, respectively, were transferred from the preceding paper, figures 2 and 3.

solution, the calcium solution, and the mixture of potassium and calcium, it will be seen that addition of calcium to potassium not only markedly increased the rate of locomotion throughout the range, but, of equal importance that it extended it far into the alkaline region.

¹ The sudden decrease to zero at pH 4.6 was probably due in great part to failure of the amoebae to attach in strongly acid solution.

This shows that calcium 0.001M very effectively antagonizes the effects of potassium 0.005N throughout the entire range of hydrogen ion concentrations between pH 4.6 and 8.0. The antagonism between potassium and calcium appears to be greater, especially in the range from pH 4.6 to 5.6 than the antagonisms between sodium and calcium. The form of the curves shows, however, that the extent of the antagonism varies greatly with hydrogen ion concentration.

It will also be seen that the variation of gel/sol ratio with hydrogen ion concentration in the potassium calcium mixture is similar to that observed in the sodium calcium mixture, with the exception that the rise in gel/sol ratio at pH 7.2 in the former was not observed in the latter mixture. Figure 2 shows that the gel/sol ratio is slightly lower for the mixture of potassium and calcium than for potassium alone, yet considerably higher throughout most of the range than for calcium. It also shows that the antagonism between potassium and calcium in reference to gel/sol ratio varies greatly with hydrogen ion concentration and that it is not so evident as it is in reference to rate of locomotion.

Sodium and potassium

Observations were made on the relation between hydrogen ion concentration and rate of locomotion in solutions containing sodium and potassium in hydrogen ion concentrations ranging from pH 4.2 to 8.0. The solutions used were made by mixing sodium and potassium phosphate buffers as described above. The observations were made the same as those in the preceding experiments. The results obtained are presented in figures 3 and 4.

Figure 3 shows that the relation between rate of locomotion hydrogen ion concentration in solutions containing potassium 0.005N and sodium 0.001N is essentially the same as that obtained with potassium 0.005N alone. This indicates that there is no antagonism between potassium 0.005N and sodium 0.001N in reference to rate of locomotion.

Figure 4 shows that the relation between hydrogen ion concentration and rate of locomotion in solutions containing sodium 0.005N and potassium 0.001N is similar to that obtained in sodium 0.005N alone with the exception that the optimum is somewhat more acid and the rates on the alkaline side of the optimum are uniformly lower in the mixture than

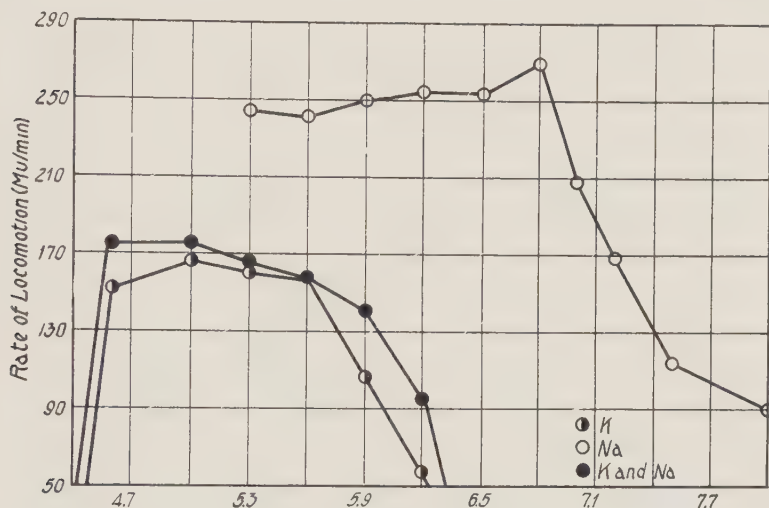


Fig. 3 Antagonism between 0.001N sodium and 0.005N potassium in reference to rate of locomotion. The curve for the solution containing sodium and potassium is based on the measurement of the rate of locomotion on an average of 12.8 specimens for a total average of 70.7 minutes in each hydrogen ion concentration tested. The curves for potassium and sodium, respectively, were transferred from the preceding paper, figures 1 and 2.

in the sodium solution alone. Thus it seems that the addition of potassium 0.001N to sodium 0.005N produces an effect on the relation between hydrogen ion concentration and rate of locomotion very similar to an increase in sodium or potassium ion concentration. This indicates that there is no antagonism between sodium 0.005N and potassium 0.001N in reference to rate of locomotion in Amoeba.

Magnesium and potassium or sodium

Observations on the relation between hydrogen ion concentration and rate of locomotion in solutions containing sodium and magnesium and potassium and magnesium were made as described above. The results obtained are presented in figure 5.

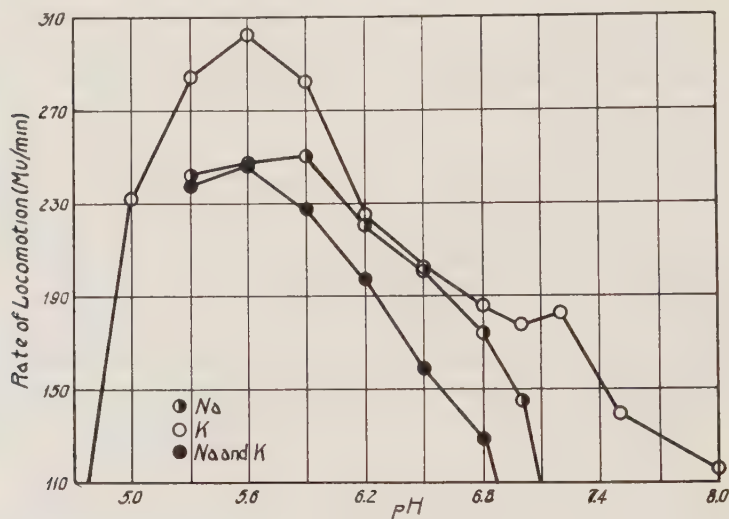


Fig. 4 Antagonism between 0.001N potassium and 0.005N sodium in reference to rate of locomotion. The curve for the solution containing sodium and potassium is based on the measurement of the rate of locomotion on an average of 17.2 specimens for a total average of 86.5 minutes in each hydrogen ion concentration tested. The curves for sodium and potassium, respectively, were transferred from the preceding paper, figures 1 and 2.

Figure 5 shows clearly that the curves describing the variation in rate of locomotion with hydrogen ion concentration in solutions of sodium 0.005N and solutions containing sodium 0.005N and magnesium 0.001N are practically identical; and that the same is true in reference to potassium and a mixture of potassium and magnesium. Thus it may be concluded that magnesium does not (at least in the concentration used) appreciably antagonize the effects of sodium or potassium on the rate of locomotion in *Amoeba*.

This is conclusive proof that the marked antagonistic action of calcium on rate of locomotion in solutions of the alkali metals is not a function of its valency but rather a specific property of calcium.

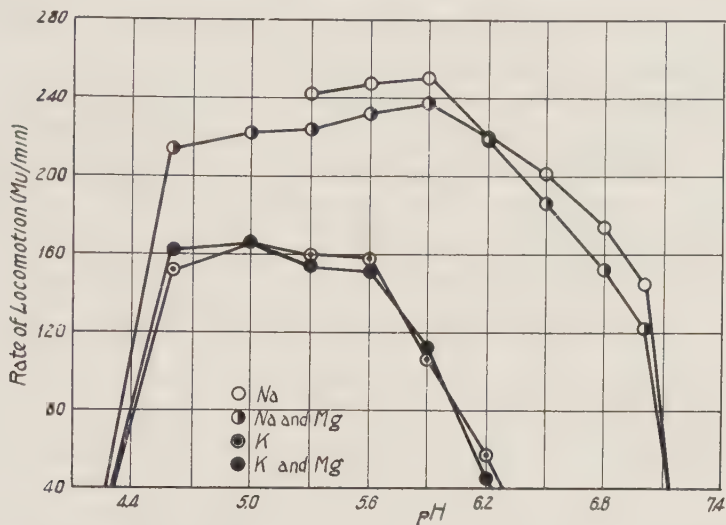


Fig. 5 Antagonism between 0.001N magnesium and, respectively, 0.005N sodium and 0.005N potassium in reference to rate of locomotion. The curve for the solution containing sodium and magnesium is based on the measurement of the rate of locomotion on an average of 14.9 specimens for a total average of 76 minutes in each hydrogen ion concentration tested; and the curve for the solution containing potassium and magnesium on the measurement of the rate of locomotion on an average of 13.5 specimens for a total average of 70 minutes in each hydrogen ion concentration tested. The curves for sodium and potassium, respectively, were transferred from the preceding paper, figures 1 and 2.

RELATION BETWEEN HYDROGEN ION CONCENTRATION AND THE EFFECT OF VARIATION IN THE PROPORTION OF DIFFERENT SALTS AND IN SALT CONCENTRATION ON ANTAGONISM BETWEEN SALTS

Hopkins ('26) and Mast and Prosser ('32) observed that *Amoeba proteus* is very much less active in neutral solutions than in solutions which are either slightly acid or slightly alkaline. Mast and Prosser maintain, however, that this does not obtain if the salt content of the solution is low and they

conclude that the relative inactivity in neutral solutions is correlated with the concentration of the salts in the solution.

In the preceding papers of this series we presented results which are in harmony with those presented by Hopkins and Mast and Prosser, but we found that they obtain only for balanced salt solutions and solutions which contain calcium and sodium or potassium, that they do not obtain for solutions which contain only sodium or potassium or calcium salts, or magnesium and sodium or potassium salts. These results indicate that the relative inactivity at neutrality in the former solutions is in some way correlated with the interaction between calcium and sodium or potassium. We, however, also obtained some evidence which indicates that the decrease in activity is not so great in strong as it is in weak salt solutions, that is, that it is correlated with salt concentration but in the reverse direction from that maintained by Mast and Prosser.

In order to obtain more information concerning this problem two sets of experiments were performed as follows: 1) The rate of locomotion and the gel/sol ratio were ascertained in three series of solutions all with constant sodium ion concentration, 0.005N, but with calcium concentrations 0.001M, 0.0005M and 0.0001M, respectively. 2) The rate of locomotion and the gel/sol ratio were ascertained in three series of solutions all with constant calcium concentration, 0.0005M, but with the sodium ion concentration, 0.010N, 0.005N and 0.001N, respectively. The observations in both sets of experiments were made in the same way as those in the preceding sections. The results obtained are presented in figures 6 and 7.

The curves in figure 6 clearly indicate that the relation between hydrogen ion concentration and rate of locomotion in the three solutions containing the three different sodium/calcium ratios produced by changing the calcium concentration is essentially the same throughout the whole range of hydrogen ion concentrations except in the region of neutrality, and that in this region, the higher the calcium concentration or the lower the sodium/calcium ratio, the lower the rate

of locomotion resulting in a median minimum rate which becomes more pronounced as the concentration of calcium increases. The curves in figure 7 indicate the same in reference to the three solutions containing the three different sodium/

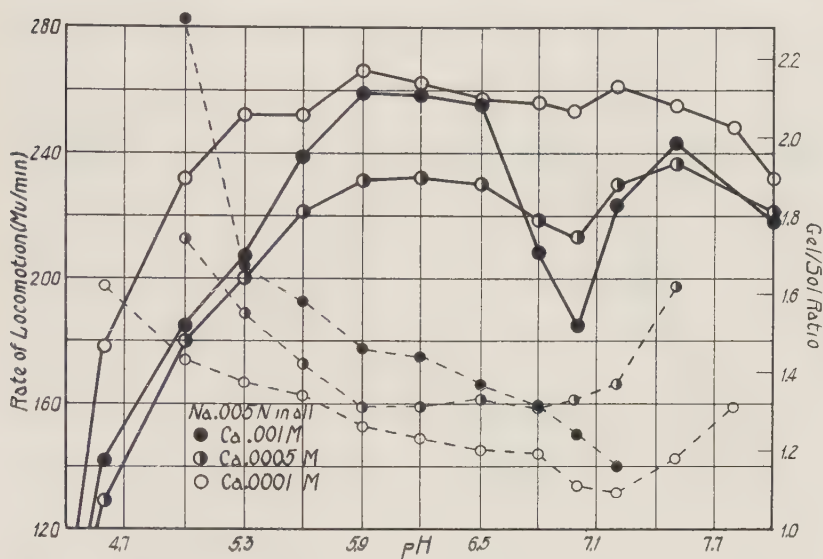


Fig. 6 The effect of adding calcium in different concentrations to 0.005N sodium solutions, on the relation between hydrogen ion concentration, rate of locomotion and gel/sol ratio. The solid curve for the solutions containing calcium 0.001M is based on measurement of the rate of locomotion, on an average of 14.75 different specimens for a total average of 89.9 minutes; that for calcium 0.0005M on an average of 18.6 different specimens for a total average of 95 minutes; and that for calcium 0.001M on an average of 20.5 different specimens for a total average of 104.6 minutes in each hydrogen ion concentration tested. The broken curve for the solution containing calcium 0.001M is based on measurement of the gel/sol ratio, on an average on 19.8 different specimens; that for calcium 0.0005M on an average of 17.9 different specimens; and that for calcium 0.0001M on an average of twenty different specimens in each hydrogen ion concentration tested.

calcium ratios produced by changing the sodium concentrations.

The results obtained in both experiments therefore show that, within the limits of the concentrations used, the lower the sodium/calcium ratio the lower the rate of locomotion at

neutrality and the greater the difference between this rate and the rates obtained at the optimum hydrogen ion concentration in alkaline and acid solutions, respectively. This together with the fact that no indication of reduction in rate

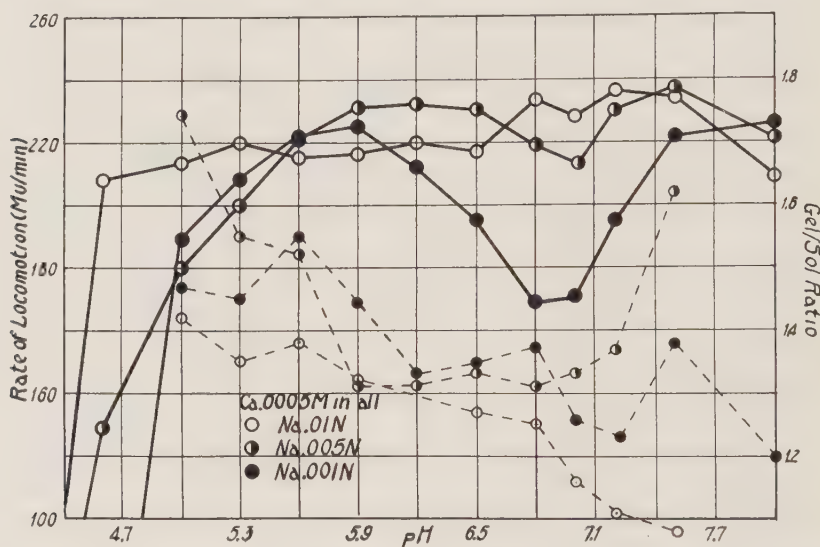


Fig. 7 The effect of adding sodium in different concentrations to 0.0005M calcium solutions, on the relation between hydrogen ion concentration and rate of locomotion. The solid curve for the solution containing sodium 0.01N is based on measurement of the rate of locomotion, on an average of 19.0 different specimens for a total average of 95.8 minutes; that for sodium 0.005N on an average of 18.6 different specimens for a total average of 95 minutes; and that for sodium 0.001N on an average of 18.9 different specimens for a total average of 95.6 minutes in each hydrogen ion concentration tested. The broken curve for the solution containing sodium 0.01N is based on measurement of the gel/sol ratio, on an average of 16.6 different specimens; that for sodium 0.005N on an average of 17.9 different specimens; and that for sodium 0.001N on an average of 18.4 different specimens in each hydrogen ion concentration tested.

of locomotion at neutrality was observed in solutions containing either of these salts alone proves conclusively that the relative inactivity observed in *Amoeba proteus* at neutrality is specifically correlated with the relation between the quantities of sodium and calcium salts present. This, of course, does not exclude the possibility of correlation with the

total salt concentration of such a nature as to eliminate the median minimum rate at neutrality in weak solutions in accord with the views of Mast and Prosser and also in strong solutions in accord with the results presented in the first paper in this series.

Figure 6 shows that the gel/sol ratio increases consistently with increase in hydrogen ion concentration, and it clearly indicates that in the hydrogen ion concentrations tested the more calcium present, i.e., the lower the sodium/calcium ratio, the higher the gel/sol ratio. The indicated increase in gel/sol ratio in the alkaline range is doubtless due to an error in measurement, owing to the accumulation of fluid under the plasmalemma and the consequent abnormal thickening of the hyaline layer as described in the preceding paper.

The results presented in figure 7 also indicate that the gel/sol ratio increases with decrease in the sodium/calcium ratio, but that those obtained in the two highest ratios are confused in some hydrogen ion concentrations. It is, however, clear that the gel/sol ratio in the lowest sodium/calcium ratio was throughout the whole range of hydrogen ion concentration tested, higher than the gel/sol ratio in the highest sodium/calcium ratio, in spite of the fact that the salt concentration was lower in the former than in the latter. The increase in total salt concentration would in itself tend to produce the reverse relations in the gel/sol ratio. This shows very clearly that the gel/sol ratio is more intimately correlated with the sodium/calcium ratio than with the salt concentration.

DISCUSSION

Gel/sol ratio

The evidence presented in the three papers of this series indicates the following in reference to the gel/sol ratio in *Amoeba proteus*:

In balanced salt solutions, in solutions containing sodium and calcium salts or potassium and calcium salts, and in solutions containing only sodium or potassium salts the gel/sol

ratio, in general, varies directly with hydrogen ion concentration.² In solutions containing only calcium it is practically independent of hydrogen ion concentration in the more dilute solutions, and probably varies inversely with the hydrogen ion concentration in the more concentrated solutions. In solutions containing calcium and sodium or potassium it is, in general, intermediate between what it is in solutions containing these salts singly.

The results presented in figures 5 and 6, paper III, indicate that the gel-sol ratio varies directly with the calcium/sodium ratio. This variation may, however, have been due to the change in total salt concentration that accompanied the change in the calcium/sodium ratio.

In balanced salt solution the gel/sol ratio varies directly with the salt concentration throughout the entire range of hydrogen ion concentration tested. In solutions of single salts the relation between gel/sol ratio and concentration is not definitely established. The results presented in figures 1 and 3, paper II, indicate that in solutions containing only calcium salts the gel/sol ratio varies directly with the salt concentration in the more acid range (pH 5 to 5.9) and inversely in the more alkaline range; and that in solutions containing only sodium salts the reverse obtains, i.e., that it varies indirectly with the salt concentration in the more acid range (pH 5 to 5.9) and directly in the more alkaline range (pH 5.9 to 6.5). There was, however, so much variability in these results that their significance is questionable.

It is obvious that a substance in the environment may influence processes which occur in a cell either by entering the cell and acting directly on substances in the cell, or by acting on the surface of the cell in such a way as to retard or facilitate the passage into or out of the cell of other substances which, owing to their presence or absence, induce alterations in internal processes.

² Some of the curves indicate inverse variation in the more alkaline solutions of these salts. It must, however, again be emphasized as in paper II, that in alkaline solutions and in toxic salt solutions in general, structural changes occurred which invalidate the results obtained in the measurement of the gel/sol ratio.

It may be assumed, then, either, 1) that the gel/sol ratio depends upon the entrance of salts into the cell and reaction between these and internal substances, and that the rate of entrance of salts varies with the hydrogen ion concentration; or, 2) that the gel/sol ratio varies with the rate of entrance of hydrogen and hydroxyl ions into the cell and reaction between these and internal substances, and that the rate of entrance of these ions varies with the concentration of the salts and the hydrogen ions; or, 3) that the gel/sol ratio depends upon the exit of substances from the cell, e.g., water, and that this depends upon the hydrogen ion concentration, and the salt concentration and the kinds of salts present. Let us now attempt to ascertain if the processes in question are in accord with any of these groups of assumptions.

If they are in accord with the first of these assumptions, the gel/sol ratio must vary directly or indirectly with the amount of salt that enters the cell and this must vary directly or indirectly with the hydrogen ion concentration. If it varies indirectly with the amount of salt that enters and this varies either directly or indirectly with the hydrogen ion concentration practically none of the results obtained are in accord with the assumptions. If it varies directly with the amount of salt that enters and this varies directly with the hydrogen ion concentration the results obtained with balanced salt solutions and some others are in full accord with the assumptions; but the assumption that the entrance of salts varies directly with hydrogen ion concentration is not in harmony with the results obtained by practically all who have investigated this problem. Moreover, the assumptions do not account for the independence or inverse variation between hydrogen ion concentration and gel/sol ratio in calcium solutions.

If they are in accord with the second of the three assumptions made above, the gel-sol ratio must depend upon the hydrogen ion concentration within the cell and this must vary with the concentration of the salts and the hydrogen ions in the surrounding medium. If it varies indirectly with the hydrogen ion concentration of the surrounding medium and

directly or indirectly with the salt concentration, few if any of the results obtained are in accord with the assumptions. If it varies directly with the hydrogen ion concentration and the salt concentration of the surrounding medium, the results obtained with balanced salt solutions and some others are in accord with the assumptions, but those obtained with calcium solutions are not. There is, moreover, no evidence which indicates that the hydrogen ion within *Amoeba* varies appreciably with variation in the hydrogen ion and the salt concentrations of the surrounding medium (Chambers, '28).

In accord with the third group of assumptions, the gel/sol ratio must vary with the rate of exit of substances from the cell, and this must vary directly or indirectly with the salt and the hydrogen ion concentration, and it must also vary with the kind of salts present in the environment. Without entering upon a detailed analysis of the correlation between these assumptions and the results under consideration, it is evident that, no matter what combination is selected, there are between them and the results inconsistencies of the same nature as those presented above.

It is consequently obvious that the results in hand cannot be consistently explained by any one of the three groups of assumptions made, and that there must be a fairly complicated interaction between the various factors involved. If this is true, the statement, without qualification, that any given factor facilitates gelation or solation is obviously so incomplete that it is without value. This conclusion is in full harmony with that reached by Mast and Prosser ('32).

Rate of locomotion

In reference to the relations between rate of locomotion and kind of salts, salt concentration and hydrogen ion concentration, the results obtained are, on the basis of any one of the groups of assumptions considered above, even less explicable than are those in reference to the gel/sol ratio. The outstanding difficulty here concerns the remarkable decrease in rate of locomotion as neutrality is approached either from the acid

or from the alkaline side. The results presented show that this decrease occurs in balanced salt solutions; that it is specifically correlated with the Na/Ca or the K/Ca ratio; that the higher this ratio within the limits of the concentrations tested, the greater the decrease; and that it does not occur in solutions containing only one salt, or in solutions containing only sodium and potassium salts, or magnesium and sodium or potassium salts.

The results show that if calcium is added to a solution containing only sodium salts, the rate of locomotion in the alkaline range increases greatly, with but little change in the acid range and in the region of neutrality; and that if sodium is added to solutions containing calcium salts, the rate decreases greatly in the region of neutrality, with but little change elsewhere.

The questions now arise as to why addition of calcium to solutions containing only sodium salts causes great increase in rate of locomotion in the alkaline range, and why addition of sodium to solutions containing only calcium salts causes great decrease in the rate in the region of neutrality.

Similar questions have arisen in reference to the bimodal curves obtained by a number of other investigators in plotting the rate of various physiological processes against hydrogen ion concentration, e.g., by Robbins ('23) and Farr ('28) in various processes in plants; by Ephrussi and Neukomm ('27) in the resistance to heat in the eggs of a sea urchin; by Hopkins ('28) in the rate of locomotion in *Amoeba*; by Mast ('28) in the rate of assumption of stellate forms in *Amoeba*; by Eisenberg-Hamburg ('29) in the rate of increase in water content in Infusoria; by Chalkley ('29) in water content and gel/sol ratio in *Amoeba*; by Chalkey ('30) in thermal death rate in *Paramecium*; by Chase and Glazer ('30) in rate of locomotion in *Paramecium*; and by Mast and Prosser ('32) in rate of locomotion in *Amoeba*.

Only a few of these investigators attempted to elucidate the phenomenon, Mast and Prosser ('32), as previously stated, concluded that it is correlated with salt concentration.

We have already considered this view. Robbins ('23) contends that the hydrogen ion concentration, at which the median minimum in the plant processes studied occurs, coincides with the isoelectric point of the principal proteins in the plant. Farr ('28) maintains, however, that this view is not tenable. He found in observations on the relation between hydrogen ion concentration and rate of growth in root hairs of collards that the hydrogen ion concentration at which the median minimum occurs varies greatly with salt concentration and he concludes that it therefore cannot be specifically correlated with the isoelectric point of any given protein in the organism.

In reference to *Amoeba*, the constancy of a median minimum rate of locomotion at pH 7.0 in various solutions indicates some fixity of mechanism determining this median minimum. This mechanism might be correlated with the behavior of the membrane in the neighborhood of an ampholyte isoelectric point near neutrality in accord with the view of Robbins ('23). But arguing against such an isoelectric point are the pertinent facts that, 1) the median minimum is lacking in solutions of single salts, though locomotion in the dilute solutions occurs at hydrogen ion concentrations in which it is usually found; 2) the difference between the maximum and the median minimum rate of locomotion depends on the sodium/calcium ratio and only slightly if at all on the total salt concentration; 3) the cations have marked effect on the rate of locomotion on the acid side of the neutral point as well as on the alkaline side; 4) the anions (so far as chloride and phosphate are concerned) have little effect down as far in the acid range as the observations were made.

We are at present unable to suggest a satisfactory explanation for this median minimum. Whatever the cause of it may be, the relation between the rate of locomotion in *Amoeba* and the factors in its environment is doubtless fairly complex, for it is probable that these factors influence locomotion in it by their action on the surface, affecting adhesiveness and other properties of the surface, as well as by their action on permeability of the surface membrane (Mast, '26).

SUMMARY

1. There is antagonism between calcium and sodium or potassium salts in reference to the effect on rate of locomotion and gel/sol ratio in *Amoeba proteus* but it varies greatly with the hydrogen ion concentration.

2. There is no antagonism between sodium and potassium salts or between magnesium and sodium or potassium salts.

3. The rate of locomotion in potassium salt solutions is in all hydrogen ion concentrations tested, increased by the addition of calcium, but the increase is much greater in solutions which are more alkaline than pH 5.6 than in those which are more acid than this, and the increase is not uniform in the more alkaline range. As the alkalinity increases from pH 5.6 the magnitude of the increase in rate increases to a maximum at pH 6.2, then decreases sharply to a minimum at pH 7.0 after which it increases sharply again.

4. The effect of addition of calcium on the rate of locomotion in sodium salt solution is similar to the effect of the addition of calcium to potassium salts except in hydrogen ion concentrations above pH 5.9 in which it actually causes decrease in rate of locomotion.

5. The effect of addition of calcium on the gel/sol ratio in sodium or potassium salt solutions varies greatly with the hydrogen ion concentration but there is no such marked change in the magnitude of this effect in the region of neutrality as there is in the effect on rate of locomotion.

6. In solutions containing calcium and sodium or potassium there is a median minimum rate of locomotion at neutrality and two maximum rates, one at pH 6.2 and one at pH 7.5, that is, the curve expressing the relation between hydrogen ion concentration and rate of locomotion in these solutions is bimodal.

7. In solutions containing only sodium, potassium, or calcium salts or only magnesium and sodium or potassium salts there is no median minimum rate of locomotion and the curves expressing the relation between rate of locomotion and hydrogen ion concentration are not bimodal.

8. The greater the concentration of calcium in relation to the concentration of sodium, that is, the lower the sodium/calcium ratio, within the limits tested, the greater the difference between the maximum and the median minimum rates of locomotion. The relative inactivity of *Amoeba proteus* at neutrality is therefore closely correlated with the sodium/calcium ratio of the solution and doubtless also with the potassium/calcium ratio. It is probably also to some extent correlated with the total salt concentration.

9. Why the effect of addition of calcium on rate of locomotion in sodium or potassium solutions is so strikingly different in neutral solutions from what it is in those which are either acid or alkaline is not clear.

10. The gel/sol ratio in solutions containing sodium and calcium varies indirectly with the sodium/calcium ratio throughout the entire range of hydrogen ion concentration tested, and it varies directly with the hydrogen ion concentration. The curves expressing these relations are not bimodal. There is no marked change in the gel/sol ratio at neutrality.

LITERATURE CITED

- CHALKLEY, H. W. 1929 Changes in water content in *Amoeba* in relation to changes in its protoplasmic structure. *Physiol. Zoöl.*, vol. 2, pp. 535-574.
- 1930 Resistance of *Paramoecium* to heat as affected by changes in hydrogen-ion concentration and in inorganic salt balance in the surrounding medium. *U. S. Public Health Repts.*, vol. 45, pp. 481-489.
- 1930 On the relation between resistance to heat and the mechanism of death in *Paramoecium*. *Physiol. Zoöl.*, vol. 3, pp. 425-440.
- CHAMBERS, R. 1928 Intracellular hydron concentration studies. I. The relation of the environment to the pH of the cytoplasm and of its inclusion bodies. *Biol. Bull.*, vol. 55, pp. 369-376.
- CHASE, A., AND O. GLAZER 1930 Forward movement of *Paramoecium* as a function of the hydrogen-ion concentration. *J. Gen. Physiol.*, vol. 13, pp. 627-636.
- EISENBERG-HAMBURG, E. 1929 Recherches comparatives sur le fonctionnement de la vacuole pulsatile chez les infusoires parasites de la grenouille et chez les infusions d'eau douce. Influence de la pression osmotique, des électrolytes et du pH. *Arch. f. Protistenkunde*, Bd. 78, S. 251-270.
- EPHREUSI, BORIS AND ALAX. NEUKOMM 1927 Résistance à la chaleur des oeufs d'oursin (*Paracentrotus lividus*, Lk.). *Protoplasma*, Bd. 2, S. 34-44.

- FARR, C. H. 1928 Studies on the growth of root hairs in solutions. IV. The pH molar rate relation for collards in calcium chloride. *Am. J. Bot.*, vol. 15, pp. 6-31.
- HEILBRUNN, L. V. 1928 The colloid chemistry of protoplasm. Berlin.
- HOPKINS, D. L. 1926 The effect of hydrogen-ion concentration on locomotion and other life processes in *Amoeba proteus*. *Proc. Nat. Acad. Sci.*, vol. 12, pp. 311-315.
- 1928 The effects of certain physical and chemical factors on locomotion and other life processes in *Amoeba proteus*. *J. Morph. and Physiol.*, vol. 45, pp. 97-119.
- MAST, S. O. 1923 Mechanics of locomotion in *Amoeba*. *Proc. Nat. Acad. Sci.*, vol. 9, pp. 258-261.
- 1926 Structure, movement, locomotion, and stimulation in *Amoeba*. *J. Morph. and Physiol.*, vol. 41, pp. 347-425.
- 1928 Factors involved in changes in form in *Amoeba*. *J. Exp. Zool.*, vol. 51, pp. 97-120.
- MAST, S. O., AND C. L. PROSSER 1932 Effect of temperature, salts, and hydrogen-ion concentration on rupture of the plasmagel sheet, rate of locomotion, and gel/sol ratio in *Amoeba proteus*. *J. Cell. and Comp. Physiol.*, vol. 1, pp. 333-354.
- PITTS, R. F., AND S. O. MAST 1933 I. Rate of locomotion, gel/sol ratio, and hydrogen ion concentration in balanced salt solutions. *J. Cell. and Comp. Physiol.*, vol. 3, pp. 449-462.
- 1934 II. Rate of locomotion, gel/sol ratio, and hydrogen ion concentration in solutions of single salts. *J. Cell. and Comp. Physiol.*, vol. 4, pp. 237-256.

VITA

Robert F. Pitts was born in Indianapolis, Indiana, on October 24, 1908. He received his elementary education in the Indianapolis grammar schools and his secondary education in the Arsenal Technical High School. He entered Butler University in 1925, and received the B.S. degree in June, 1929.

In the fall of 1929 he entered the Johns Hopkins University as a student in zoölogy. He was assistant in general biology, 1929-1930, and in comparative anatomy, 1930-1931. He held the Adams T. Bruce fellowship, 1931-1932.

